RESPONSES MADE BY THE SALT MARSH TELEOST *CYPRINODON VARIEGATUS* (ATHERINOMORPHA: CYPRINODONTIDAE) TO LIFE IN A VARIABLE SALINITY ENVIRONMENT

By

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Cyprinodon variegatus, a common coastal resident of the western Atlantic Ocean and Gulf of Mexico, lives in ambient salinities ranging from freshwater to 142 ppt. Fish used in this study were obtained from a Gulf of Mexico salt marsh near Cedar Key, Florida. In a steady-state experiment, routine metabolic rate (RMR) and critical oxygen tension (P_C) were determined at salinities ranging from 0 to100 ppt. Salinities between 0 and 40 ppt had little influence on RMR or P_C. However, at salinities above 40 ppt, RMR declined, and P_C increased. The reduction in RMR and rise in P_C correlates with a reduced ability of *C. variegatus* to osmoregulate effectively at high salinities. The variations in RMR and P_C at high salinities suggests that *C. variegatus* responds by reducing energy expenditures, effectively increasing the time that individuals can tolerate hypersaline conditions. The metabolic patterns of *C. variegatus* as influenced by simulated tidal changes in salinity were then measured, with RMR unaffected by changes in salinity between 2 and 40 ppt. However, at extremely high or low salinities metabolism was affected by changes in ambient

salinity. Individuals of *C. variegatus* responded to fluxes at salinity extremes by reducing general activity and energy expenditures—essentially waiting for conditions to return to normal, where they responded by increasing metabolic activity. *Cyprinodon variegatus* efficiently regulated plasma osmolality, even when fishes were exposed to large fluctuations in salinity. However, prior exposure to salinity fluctuations did impart an osmoregulatory advantage. Fish previously exposed to large salinity fluctuations regulated plasma osmolality better than fish that previously experienced no or small changes in salinity. Increasing salinity had a greater impact on osmoregulation than did decreasing salinity. Salinity also had a significant effect on blood oxygen carrying capacity in *C. variegatus*, although differences were only noted at the very highest (60 - 80 ppt) and lowest (0 ppt) salinities tested. Oxygen carrying capacity was highest in the group acclimated to 0 ppt. Erythrocyte count was the most consistent indicator of the influence of salinity on blood oxygen, with hematocrit the least consistent measure.

CHAPTER 1
INTRODUCTION

Salinity is a crucial physicochemical factor that exerts an important influence on aquatic life, particularly on estuarine and salt marsh organisms that are exposed to unpredictable salinity fluctuations diurnally and seasonally. Salt marshes are intertidal beds of rooted vegetation that are alternately flooded and exposed by rising and falling tides. Vegetation on higher ground develops a complex network of branching channels through which water, nutrients, and aquatic organisms move during the tidal cycle. Intertidal salt marshes are inhospitable yet interesting environments due to their unstable water levels and to the extreme variability of their physical, chemical, and biological processes (Cooper, 1974; Adam, 1990; Allen and Pye, 1992).

Factors that may influence salinity in salt marshes include precipitation, wind, frequency and extent of tides, shoreline height, and coastal topography (Remane and Schlieper, 1971; Wheatly, 1988). Due to the harshness of the physical environment, salt marshes tend to have low species diversity, but often high abundance of selected species (McClusky, 1989; Dunson and Travis, 1994).

Whereas variable salinity habitats are also characterized by fluctuations in other environmental factors, such as temperature, dissolved oxygen, and pH (Vernberg, 1983; Wheatly, 1988), the distribution and abundance of fishes in these habitats is largely determined by salinity (McClusky, 1989; Davenport and Sayer, 1993; Gill and Potter, 1993). Not surprisingly, animals respond to fluctuations in salinity in complex ways. Salinity affects osmoregulation, ventilation, metabolism, acid-base balance, growth, reproduction, development, and other biological processes (Wheatly, 1988). Some of the primary responses of teleosts to changes in salinity are reviewed here.

Avoidance is the first line of defense to variable salinity conditions. If behavioral responses do not sufficiently minimize exposure to variable salinities, aquatic organisms must rely on physiological and biochemical responses to tolerate environmental changes (Beitinger and McCauley, 1990). This may be by passive tolerance (osmoconforming) or active osmoregulation (Truchot, 1987; McClusky, 1989). Only fishes that are capable of osmoregulation can tolerate wide changes in salinity, and these euryhaline teleosts will be the focus of the rest of this review.

The basic patterns of osmoregulation in fishes have been extensively reviewed in recent years (Parvatheswararao, 1970; Eddy, 1982; Evans, 1984; Zadunaisky, 1984; Karnaky, 1986; Foskett, 1987; Pisam and Rambourg, 1991; Ventrella et al., 1992; Evans, 1993; McCormick, 1994; Wood and Marshall, 1994). Euryhaline teleosts regulate their blood osmolality at about one-third the concentration of seawater (35 ppt), and thus face severe osmotic problems whether in freshwater (0 ppt) or seawater. Body fluids of a teleost in freshwater are hyper-osmotic to the external environment, whereas in seawater they are hypo-osmotic. Thus, euryhaline fish possess mechanisms for osmoregulating in both hyper-osmotic and hypo-osmotic conditions.

Teleosts in seawater are susceptible to a loss of body water to the external environment, and balance water loss by actively drinking large amounts of seawater. However, both water and salts are absorbed together across the gut. Ingested excess salts are actively excreted, divalent ions mostly in urine and feces, and monovalent ions by the gills.

Active excretion of salts by the gills takes place via chloride cells (Zadunaisky, 1984; Karnaky, 1986; Foskett, 1987; Pisam and Rambourg, 1991). Chloride cells in seawater-acclimated fish are located at the base of the secondary gill lamellae, and are large, columnar-shaped cells usually extending from the basal epithelium to the external environment. They are characterized by numerous mitochondria (for this reason they are often referred to as "mitochondria rich cells") and an extensive tubular reticulum continuous

with the basolateral membrane (Pisam and Rambourg, 1991). The transport enzyme Na^+,K^+-ATPase is restricted to this tubular system and to the basolateral membrane. This ion pump creates a large Na^+ gradient (low in the chloride cell cytoplasm) which drives a NaCl co-transporter by which Cl^- enters the cell. The Cl^- accumulates sufficiently in the cell such that it is able to exit to the external environment across the apical membrane, with Na^+ following passively down its electrochemical gradient between adjacent chloride cells (Evans, 1993; McCormick, 1994; Wood and Marshall, 1994).

In freshwater, teleosts continuously face an efflux of salts and an influx of water. Hence, their osmoregulatory response is to actively transport salts from the external environment via the gills, to avoid drinking water, and to excrete copious amounts of dilute urine. Our present understanding of the mechanisms involved in the uptake of Na^+ and Cl^- from the environment in freshwater teleosts is somewhat unclear and incomplete. It appears that Cl^- is actively taken up, with Na^+ uptake being a passive process. Whether this takes place via a freshwater-type chloride cell, or through interactions with acid-base balance is unclear, as evidence exists for both (Wood and Marshall, 1994).

What is unique about osmoregulation in euryhaline fish is not that they possess a structurally distinct osmoregulatory mechanism, but that they have the ability to function efficiently in variable salinities. They must not only be able to hypo- and hyper-osmoregulate, but they must be able to alter their pattern of osmoregulation quickly if they live where salinity rapidly fluctuates.

Osmoregulation is controlled largely by the endocrine system. In teleosts, hormones known to exert osmoregulatory effects include prolactin, growth hormone, cortisol, angiotensin II, arginine vasotocin, atrial natriuretic peptide, thyroid hormones, urotensins, vasoactive intestinal peptide, insulin, calcitonin, catecholamines and sex steroids (Jenkins, 1981; Bern and Madsen, 1992; Leloup and Lebel, 1993; Sakamoto et al., 1993; Takei, 1993; McCormick, 1994). The hormone most involved with osmoregulation under freshwater conditions is prolactin, with arginine vasotocin, urotensin II, catecholamines, and atrial

natriuretic peptide also playing roles (Hirano et al., 1987; Bern and Madsen, 1992; Takei, 1993). Control of osmoregulation in seawater is largely via the effects of cortisol and growth hormone, together with thyroid hormones, angiotensin II, vasoactive intestinal peptide, atrial natriuretic peptide, and urotensin I (Balment et al., 1987; Bern and Madsen, 1992; Takei, 1993). Although the exact mechanisms and interactive effects of many of these hormones are unclear, it is well established that both rapid and long term control of osmoregulation under hyper-osmotic and hypo-osmotic conditions is mediated via the endocrine system.

Osmoregulation is not the only physiological process affected by salinity. Salinity adaptation is a complex event that involves a number of physiological and behavioral responses, including energetics. Many studies have shown that salinity influences metabolism of fishes, but little agreement exists with respect to the magnitude or direction of these effects (Nordlie, 1978; Febry and Lutz, 1987; Nordlie et al., 1991; Swanson, 1991; Morgan and Iwama, 1991; Kirschner, 1993). One reason for a lack of agreement among studies is that many have focused on the energetic costs associated with osmoregulation, while ignoring other related physiological and behavioral processes. For example, many studies have used measured differences in metabolism at different salinities to represent differences in osmoregulatory costs. The rationale is that osmoregulation is an energetically costly event, with the energetic costs being related to the osmotic gradient between the fish and its environment (e.g., Madan Mohan Rao, 1968; Muir and Niimi, 1972; Furspan et al., 1984; Zadunaisky, 1984). However, this is probably an oversimplification, since other factors, such as activity, food intake, and permeability changes may also be influenced by salinity, and in turn, affect metabolism (Swanson, 1991). For this reason metabolic measurements represent the overall costs associated with living in a particular salinity environment, with comparisons among salinities reflecting these total costs, not simply the cost of osmoregulation.

Several attempts have been made to categorize the patterns of metabolic responses of teleosts to altered salinities (Kinne, 1967; Remane and Schlieper, 1971; Nordlie, 1978; Morgan and Iwama, 1991). One commonly observed pattern is that the lower rates of metabolism in response to salinity are associated with environments for which the species (and life stage) are presumably best adapted for, and in which they are normally found. Teleosts that inhabit widely variable salinity environments are uniquely characterized by having metabolic rates that are constant over a wide range of salinities. This allows euryhaline teleosts to avoid the large metabolic costs normally associated with physiological adjustment to changing salinity.

Another physicochemical parameter that may act synergistically with salinity is dissolved oxygen. The dissolved oxygen content of many bodies of water is subject to large natural fluctuations. This is especially true in shallow water, such as salt marshes, where chronic and/or periodic hypoxia may be common (Renaud, 1985; Dejours, 1987; Toulmond, 1987; Graham, 1990). Variations in oxygen can dictate the distribution of some species in aquatic ecosystems (Boutilier, 1990). Changing environmental salinity may directly influence respiratory function by affecting both the oxygen solubility in water pumped over the gills and the solubility of gases dissolved in plasma. Changes in the ionic composition of the body fluids could also interact with oxygen to influence tolerance to variable salinity conditions (Truchot, 1987).

Exposure of fish to reduced oxygen tensions initiates physiological responses mostly directed at increasing the amount of oxygen available to the tissues (Boutilier et al., 1988). The transfer of oxygen from the environment to the tissues can be characterized as a series of processes. Gill ventilation is the first step, followed in turn by branchial diffusion, blood oxygen transport, and diffusion into the tissues (the last of which, due to a lack of available information, will not be discussed further) (Perry and McDonald, 1993).

Gill ventilation is a function of the frequency and depth of breathing, and is normally increased when low oxygen tensions are encountered. While ventilation does not

appear to be directly limiting to the uptake of oxygen, substantial cost is involved with increased ventilatory pumping, which ultimately means that any additional oxygen acquired is used to fuel the ventilatory apparatus itself (Boutilier et al., 1988; McMahon, 1988; Cameron, 1989; Perry and McDonald, 1993).

Two processes are largely available to increase branchial oxygen diffusion: increases in functional gill surface area and increases in the mean water to blood oxygen partial pressure gradient. This tradeoff is particularly important in regard to salinity, as fish in waters of low oxygen tension must balance the advantage of maximizing branchial oxygen diffusion with a disadvantage in osmoregulation due to the accompanying increases in ion and water exchange (Perry and McDonald, 1993). Increasing blood gas transport is likely the primary route used by most fish to increase the amount of oxygen delivered to the tissues. Oxygen transport by the blood in teleosts depends on the respiratory pigment hemoglobin. Blood oxygen transport is normally increased by increasing the concentration of hemoglobin, increasing the number of erythrocytes in circulation, and/or by adjusting the affinity of hemoglobin for oxygen (Davis, 1975; Wells et al., 1989; Jensen et al., 1993; Perry and McDonald, 1993).

All of the processes described above can be modified to optimize oxygen transport under a variety of environmental conditions. One additional strategy that can be utilized in conjunction with the above is the lowering of metabolism in concert with reductions in oxygen. This potentially minimizes the impact of the lowered oxygen tension, but also reduces aerobic metabolism and therefore the amount of energy available for physiological processes.

Most fish would be described as metabolic oxygen regulators, as they maintain a constant metabolic rate over a range of oxygen tensions extending downward from atmospheric levels to some low level that has been defined as the critical oxygen tension (P_C). Below the P_C, metabolism is dependent upon oxygen tension, and decreases linearly with decreases in oxygen. The P_C was probably first documented by Hall (1929), but was

not considered an important index for fishes until formalized by Fry (1947). However, this is an extremely important variable relating to habitat selection and overall metabolic patterns of fishes, and calculations of the P_c have since been made for a number of species under a variety of environmental conditions.

Experimental Animal

The subject of this study was the sheepshead minnow, *Cyprinodon variegatus*. *Cyprinodon variegatus* is a member of the family Cyprinodontidae, a large and diverse family containing over 650 species in 80 genera (Parenti, 1981; Parker and Kornfield, 1995; see Table 1-1). Members of this family are found in fresh, brackish, and salt water, and distributed pantropically as well as throughout North America. *Cyprinodon variegatus* is the type species for both the genus *Cyprinodon* and the family Cyprinodontidae.

The genus *Cyprinodon* comprises a group of approximately 30 species of small, oviparous fishes commonly known as pupfishes. They exhibit remarkable tolerance to harsh environmental conditions (Miller, 1981) occurring throughout North and Central America, the Caribbean Sea, and Venezuela (Darling, 1976; Turner and Liu, 1977; Parenti, 1981; Duggins et al., 1983; Barus and Wohlgemuth, 1993). The genus is characterized by limited genetic divergence and few unique alleles, even in morphologically distinct species (Kodric-Brown, 1989). Much of the research on the genus has focused on species inhabiting North American deserts, where the largest concentration of species occurs. Most of these desert species have small, allopatric distributions (Miller, 1981; Duggins et al., 1983). Members of this genus also occur in the coastal brackish and marine waters of eastern North America, where *C. variegatus* is the dominant pupfish species.

Cyprinodon variegatus is the only pupfish species having an extensive geographic range. It is found along the Atlantic coast from Massachusetts to the Florida Keys, and throughout the Gulf of Mexico. A disjunct population occurs along the Yucatan peninsula (Johnson, 1974; Darling, 1976; Duggins et al., 1983). Populations are also located in the

Table 1-1. Phylogenetic classification of the cyprinodontiform fishes (modified from Parenti, 1981).

Order Cyprinodontiformes

- Suborder Aplocheiloidei
- Suborder Cyprinodontoidei
  - Section 1
    - Family Profundulidae
  - Section 2
    - Division 1
      - Family Fundulidae
    - Division 2
      - Superfamily Poecilioidea
        - Family Anablepidae
        - Family Poeciliidae
      - Superfamily Cyprinodontoidea
        - Family Goodeidae
        - Family Cyprinodontidae
          - Subfamily Cyprinodontinae
            - Tribe Orestiini
              - Genus *Orestias*
              - Genus *Kosswigichthys*
              - Genus *Aphanius*
            - Tribe Cyprinodontini
              - Genus *Cyprinodon*
              - Genus *Megupsilon*
              - Genus *Jordanella*
              - Genus *Floridichthys*
              - Genus *Cualac*

Bahamas, West Indies, and Cuba (Duggins et al., 1983). *Cyprinodon variegatus* has also been introduced into several areas, where they have negatively impacted native pupfish species (Echelle and Echelle, 1987; Echelle and Connor, 1989; Kodric-Brown, 1989; Wilde and Echelle, 1992). Genetic variability within *C. variegatus* is as large as the amount of genetic divergence within the entire genus *Cyprinodon*, with most of this genetic divergence occurring in populations north of Cape Hatteras—very little genetic divergence is displayed among southern populations (Darling, 1976; Schwartz et al., 1990).

Cyprinodon variegatus is a numerically dominant and ecologically important species throughout most of its range, especially in salt marsh and estuarine waters (Kilby, 1955; Simpson and Gunter, 1956; Relyea, 1975; Naughton and Saloman, 1978; Subrahmanyam and Coultas, 1980; Stout, 1985; Nelson, 1992; Ross and Doherty, 1994). Due to its importance, *C. variegatus* has been an important research animal in diverse disciplines. These include investigations on the species behavior (Itzkowitz, 1974; Itzkowitz, 1978; Mettee and Beckham, 1978; Itzkowitz, 1981, Dwyer and Beulig, 1991), ecology (Doll and Bast, 1969; Martin, 1970; Martin, 1972; Able, 1976; Harrington and Harrington, 1982; Fyfe, 1985; Shipley, 1991; Avila et al., 1992; Wright et al., 1993), evolution (Elder and Turner, 1994), life history (Warlen, 1964; De Vlaming et al., 1978; Berry, 1987; Able, 1990; Echelle and Echelle, 1994), physiology (Martin, 1968; Karnaky et al., 1976; Subrahmanyam, 1980; Nordlie, 1985; Barton and Barton, 1987; Nordlie, 1987; Peterson and Gilmore, 1988; Nordlie and Walsh, 1989; Peterson, 1990; Price et al., 1990; Nordlie et al., 1991; Dunson et al., 1993), and reproduction (Raney et al., 1953; Warlen, 1964; Berry, 1987; Kodric-Brown, 1987; Conover and DeMond, 1991). The species has also been used in voluminous toxicology experiments because of its extreme hardiness (e.g., Schimmel and Hansen, 1975; Hawkins et al., 1984; Battalora et al., 1985; Linton, 1992).

Cyprinodon variegatus has been called "the toughest fish in North America" (Gunter, 1967) due to its extreme tolerance of harsh environmental conditions. It is found in waters ranging from freshwater (Ager, 1971; Johnson, 1974; Kushlan, 1980) to salinities of

142 ppt (Simpson and Gunter, 1956), although it typically inhabits brackish water and coastal salt marshes. *Cyprinodon variegatus* is tolerant of temperatures ranging from about 1 °C (Berry, 1987), to 41 °C (Strawn and Dunn, 1967), and oxygen levels approaching anoxia (Odum and Caldwell, 1955). Thus, it is an exceedingly useful experimental subject for studying how teleost species respond to harsh environmental conditions.

Although certain organisms can withstand greater changes in environmental conditions than others, the ability to respond to natural environmental changes is a basic characteristic of all living systems. Unfortunately, the terminology used to describe these responses is not uniform. Various researchers have attempted to define the terms adaptation, acclimation, acclimatization, and accommodation (e.g., Prosser, 1955; Kinne, 1962; Prosser, 1975; Smit, 1980; Fontaine, 1993). I will use the term "adaptation" in its broadest sense, defining it as a modification of the characteristics of an organism that facilitate an enhanced ability to survive and reproduce in a particular environment. In this way I recognize that adaptations involve both genetic and physiological (phenotypic) components, while not attempting to separate these components from one another. The term acclimation will be used as defined by Prosser (1975), where compensatory changes are measured following changes in single environmental variables.

Questions

This study was designed to examine some of the costs to *C. variegatus* associated with living in variable salinity environments. Specifically, I asked the following questions: (1) What are the metabolic costs associated with different ambient salinities? (2) How does salinity influence the energetic response at low oxygen tensions? (3) What is the osmoregulatory response to variable salinity environments? (4) How does salinity influence blood oxygen levels? These questions were tested by the following: measurement of metabolism in *C. variegatus* fully acclimated to a wide range of experimental salinities; measurement of the critical oxygen tension in *C. variegatus* fully acclimated to the same

range of salinities; measurement of metabolism prior to, and following, simulated tidal changes in salinity; monitoring of plasma osmolality in *C. variegatus* exposed to a group of different cycling salinity regimes; and measurement of hemoglobin concentration, erythrocyte count, and hematocrit in *C. variegatus* acclimated to a wide range of ambient salinities.

Study Site

Fish used in this study were collected from tidal marshes of the Gulf of Mexico near Cedar Key, Florida. The shore in the Cedar Key area is classified as a zero energy sector in which wave energy is dampened over the broad, shallow limestone plateau of the Gulf of Mexico bottom (Stout, 1985). This results in a wide intertidal zone along the coast. Furthermore, the coastal physiography is extremely diverse due in large part to irregularities in the shore line of the mainland, to the presence of numerous islands and oyster bars in the tidal area, and to the maze of intertidal and subtidal creeks and channels (Kilby, 1955). No significant sediment sources are found in this area, and tides occur on a semi-diurnal basis. The dominant emergent vegetation in the area is *Spartina alterniflora*, with the salt marshes dominated by *Juncus roemerianus*. Fish communities of the *Juncus* marsh are dominated by atheriniforms, with *C. variegatus*, *Fundulus similis*, and *Poecilia latipinna* making up 50-90% of the catch throughout most of the year (Kilby, 1955; Simpson and Gunter, 1956; Stout, 1985; pers. obs.).

CHAPTER 2
INFLUENCE OF ENVIRONMENTAL SALINITY ON ROUTINE METABOLIC RATE AND CRITICAL OXYGEN TENSION OF *CYPRINODON VARIEGATUS*

Introduction

Most fishes are capable of tolerating only a narrow range of salinities. However, some fishes live in areas that experience frequent variations in salinity. These euryhaline species possess important physiological and behavioral mechanisms that enable them to survive in variable salinity environments. One such fish is the sheepshead minnow, *Cyprinodon variegatus*. This species ranges along most of the Atlantic coast of the U.S., throughout the Gulf of Mexico, and disjunctly along the Yucatan peninsula (Johnson, 1974; Darling, 1976; Duggins et al., 1983). It typically inhabits brackish water coastal salt marshes that undergo frequent salinity fluctuations. *Cyprinodon variegatus* is capable of tolerating salinities ranging from 0 ppt (Ager, 1971; Johnson, 1974; Kushlan, 1980) to 142 ppt (Simpson and Gunter, 1956).

This study was designed to examine the metabolic response of *C. variegatus* over a variety of environmental salinities. Whereas energetic responses to a number of variables including temperature, body mass, oxygen, and activity level have been well studied, the influence of salinity on metabolism of fishes has received less attention. Most previous studies have found that salinity does affect the energetics of fishes. Unfortunately, the magnitude and/or direction of these effects are equivocal (Febry and Lutz, 1987; Morgan and Iwama, 1991; Nordlie et al., 1991; Swanson, 1991). This has led several authors to categorize the general patterns of metabolic responses to altered salinities (e.g., Nordlie, 1978; Morgan and Iwama, 1991).

One general pattern of fishes that inhabit variable salinity environments is a stable metabolic rate over a range of salinities, with the range of salinities most commonly tested between freshwater (0 ppt) and seawater (35 ppt) (Morgan and Iwama, 1991). Physiological stability enables such fish to have a euryhaline existence that is unfettered by large metabolic costs associated with adjustment to salinity change. However, there are relatively few studies of the metabolic response of euryhaline fishes over an even wider range of salinities that they encounter in their natural habitats.

Fish metabolism is also strongly influenced by the partial pressure of oxygen (PO_2). Metabolism of fishes is independent of PO_2, as they maintain a constant metabolic rate over a range of PO_2 extending downward from high atmospheric levels to some lower level defined as the critical oxygen tension (P_c). Below the P_c (conformation region), metabolism depends on oxygen tension and decreases linearly with decreases in oxygen (Fry, 1947). Oxygen consumption declines at the P_c because the gas exchange system can no longer supply both the extra demands of the respiratory system and the oxygen demands of the tissues (Hughes, 1964). Since P_c is a useful metabolic parameter, calculations of the P_c have been made for a number of fishes under a variety of environmental conditions (e.g., Hall, 1929; Tang, 1933; Ultsch et al., 1978; Ott et al., 1980; Subrahmanyam, 1980; Donnelly and Torres, 1988; Rantin et al., 1992; Nonnotte et al., 1993). However, the influence of ambient salinity on the P_c has not been specifically studied. This is surprising since salinity and oxygen are important abiotic factors that may act synergistically in affecting metabolism.

This study examined the influence of a wide range of environmental salinities on routine metabolic rate (RMR) and P_c in *C. variegatus*. I hypothesized that both RMR and P_c would be unaffected by alterations in salinity over the range of salinities commonly encountered in natural habitats of *C. variegatus*. Salinities outside this range, but within the range known to be tolerated, were predicted to cause increases in both parameters.

Methods

Fish used in this study were obtained by seining canals and ditches in the salt marsh near Cedar Key, Florida (Gulf of Mexico). Specimens were transported back to the laboratory in 19 L carboys containing water from the collection site. Upon arrival at the laboratory, individuals were held overnight in this water with constant aeration. The following day, fish were transferred into holding tanks (75 to 114 L aquaria) maintained at the salinity at which fish were captured, and treated prophylactically for 7-14 days in a 5 mg L^{-1} solution of Acriflavine®. Following treatment, groups of approximately 10 fish were placed into experimental (38 L aquaria) tanks containing water at a salinity within 10 ppt of that in which they were collected. Both holding and experimental tanks were equipped with undergravel filtration and constant aeration, and were maintained in rooms on a 12:12 light:dark cycle. Fish were fed Tetramin® flake food once each day. All experimental aquaria were located in a constant temperature environment room that maintained aquaria at 20 ±1 °C.

Experimental aquaria were used to acclimate fish to salinities ranging from 0 ppt to 100 ppt (0 to 2860 mOsm kg^{-1}). The initial acclimation period was 14 days, after which fish were either used in a metabolic trial or were transferred to the next higher or lower salinity in the series. Salinity changes were in steps of 5 ppt, with smaller increments used to acclimate fish to 0 ppt. This procedure was repeated until determinations had been made at all experimental salinities. Water used in the freshwater acclimation was obtained from wells in Alachua and Levy counties, Florida (mean conductivity = 360 $\mu S\ cm^{-1}$). Experimental salinities greater than freshwater but less than full seawater were prepared by diluting filtered Atlantic Ocean seawater (obtained from the C.V. Whitney Laboratory of the University of Florida, Marineland, Florida) with appropriate quantities of deionized water. Salinities greater than 35 ppt were produced by supplementing seawater with appropriate

amounts of synthetic sea salt (Instant Ocean®). Salinities were monitored daily with an AO® temperature-compensated refractometer, and adjusted as necessary.

Metabolic determinations were carried out in sealed, opaque flasks ranging in volume from 0.6 to 1.175 L, with the volume selected based on the size of the fish being tested. Rate of oxygen consumption was used to measure metabolism, with flasks being used as closed respirometers. Measures of metabolism were considered to be routine metabolic rates, as animals were sequestered in such a way as to minimize, but not eliminate activity (Winberg, 1956; Fry, 1957). Experiments were performed with post-absorptive fish in a resting state, but fish were unconstrained and capable of spontaneous motor activity (Winberg, 1956; Fry, 1957). For a relatively inactive species such as *C. variegatus* (compared to actively swimming salmonids, for example), RMR is perhaps the most appropriate measure of metabolism, as it more closely reflects normal activity patterns of the fish than does either active or standard metabolic rate.

Each flask was sealed with a rubber stopper through which two hypodermic needles (No. 18) were inserted. Each needle was fitted on the inside with catheter tubing, one with a piece long enough to reach the bottom of the flask, the other half this length. A 25 ml plastic syringe filled with water at the salinity of the experimental aquarium was inserted into the needle fitted with the short piece of catheter tubing, while an empty 10 ml syringe was inserted into the other needle. A 1 ml water sample was drawn into the empty syringe for each determination of PO_2. As each water sample was withdrawn, an equal volume of water was injected from the filled syringe into the flask. This method of sampling, along with minor movements by the fish, effectively stirred the water in the flask (Nordlie, pers. comm.). Determinations of PO_2 were made with a Radiometer® oxygen electrode connected to a Radiometer® PHM 71 acid-base analyzer.

Measurements of the rate of reduction in PO_2 were made at 0.5 h intervals, and continued until fish had depleted the oxygen level to approximately 20 mm Hg (generally 5 to 9 h). Following the final PO_2 determination, each fish was removed from its flask, damp-

dried and weighed to the nearest 0.01 g. All metabolic determinations were made between 0700 and 1900 hours, and fish were not re-used in other metabolic trials.

To ensure that fish were post-absorptive at the time of testing, food was withheld from experimental aquaria for 24 h prior to beginning a metabolic reading. Respirometers were filled with water at the salinity of the experimental aquaria, and placed in a water bath maintained at 20 ±1 ^{o}C. The entire metabolic apparatus was located in a small, semi-darkened room in which no other activity took place. In order to allow time for the fish to adjust to the respirometers, individuals were placed into the flasks (with constant aeration) 12 to 16 h before beginning a trial. At the beginning of the metabolic trial, aerators were removed and the flask was sealed.

Calculation of oxygen saturation values for the experimental conditions (taking into account salinity, temperature, relative humidity, and barometric pressure) were made using the equations of Truesdale et al., 1955 and both RMR (mg O_2 h^{-1}) and P_c (mm Hg) were calculated for each fish. Data used for calculation of metabolic rates were limited to values obtained while the PO_2 in the respirometer was greater than 100 mm Hg, in order to ensure that these calculations were made at oxygen tensions well above the P_c. All data were used for calculation of the P_c. Determination of the P_c was made using a BASIC program to calculate the critical point (Yeager and Ultsch, 1989). Following recommendations by Yeager and Ultsch (1989), data for each fish were first plotted to ensure that the relationship was a two-step function, following which the midpoint approximation was used to calculate the P_c.

Oxygen consumption is strongly influenced by body mass, so RMR values were mass-adjusted using an analysis of covariance (ANCOVA). Log mass-independent RMR was used as the dependent variable and log mass as the covariate. Least square means derived from the ANCOVA were used as adjusted RMR values. It was not possible to perform an ANCOVA for the P_c values, so calculations of P_c were mass-corrected to the value of the average mass (3.13 g) of all individuals used in this study. The exponent

describing the relationship between mass and metabolism for *C. variegatus* (Nordlie et al., 1991), $MR = kW^{0.68}$, was used to correct oxygen consumption rates. Values were corrected following the relationship $MR_C = (W_O^{0.32})(3.13^{-0.32})(MR_O)$, where MR_C is the mass-corrected oxygen consumption, W_O is the observed mass, and MR_O is the observed oxygen consumption at mass W_O (Ultsch et al., 1978; Cech, 1990). Statistical analyses follow procedures outlined in Winer et al., (1991) and Sokal and Rohlf (1995). All statistical analyses were one way tests using the Tukey-Kramer post hoc comparison ($p = 0.05$), and values are given throughout as means ± standard error of the mean (se).

Field Measurements

Salt marsh habitats are widely considered to experience unpredictable and fluctuating abiotic conditions. However, actual physicochemical measurements are infrequently reported. To address this issue, field measurements were made at four sites in the Cedar Key area over a one year period. Whenever possible, measurements at each site were taken both at the surface and on the bottom (generally 1-1.5 m deep). Dissolved oxygen, salinity, and temperature were measured one to three times each month between 0700 h and 1700 h, for a total of 19 dates between June 1990 and June 1991. Sites 1, 2, and 3 were located deep in the salt marsh where *C. variegatus* was routinely collected. These sites were located in close proximity to one another (< 10 m apart), and were interconnected. Unlike many locations in the salt marsh, these sites were never completely isolated from one another or from connections to the Gulf of Mexico, even during the lowest tides. Site 4 was located directly on the Gulf of Mexico, in the town of Cedar Key, approximately five km from sites 1, 2, and 3. Although *C. variegatus* is present at site 4, collections were not made at this location. These field measurements were not intended to indicate the complete ranges of oxygen, salinity, or temperature experienced by organisms living within the salt marsh. Thus, the actual ranges of physicochemical conditions experienced by *C. variegatus* are most likely greater than reported here. However, these values are a subset of the conditions

experienced by salt marsh inhabitants, and give an indication of some of the variability in the measured physicochemical parameters.

Results

Routine Metabolism

Mean RMR was calculated for each fish and organized by salinity groups. Mean values for unadjusted and adjusted RMR (from ANCOVA) are given in Table 2-1, and adjusted RMR are plotted against ambient salinity in Figure 2-1.

In the range of ambient salinities between freshwater and 40 ppt, adjusted RMR values were highest at 2 ppt and 40 ppt, being slightly lower and roughly equivalent at the other measured salinities in this range. At salinities greater than 40 ppt, there was a progressive decline in adjusted RMR. Overall, adjusted RMR ranged from a maximum of 0.97 mg O_2 h^{-1} at 2 ppt, to a low of 0.64 mg O_2 h^{-1} at 100 ppt, representing a 66% decline. This decline corresponds with a decreased ability to regulate plasma osmolality at elevated salinities (Figure 2-2; plasma osmolality data are from Nordlie, 1985).

A multiple linear regression analysis was used to generate a predictive model for describing the effects of both salinity and mass on metabolism. In this model, Log body mass (Log W; in g) and salinity (S; in ppt) were used as independent variables, and Log mass-independent RMR (Log MR; in mg O_2 h^{-1}) as the dependent variable. The equation that best described this relationship is

$$\text{Log MR} = -0.296 + 0.548\ (\text{Log W}) - 0.001\ (\text{S})\ (F_{2,110} = 85.445;\ P < 0.0001)$$

This model described 62% of all variability about the mean, and the random distribution of the residuals suggests an absence of significant relationships that might have biased the analysis.

Table 2-1. Relationships of routine metabolism (RMR), critical oxygen tension (P_C), and slope in the conformation region at a series of ambient salinities. Values are given as means ± se.

Ambient Salinity (ppt)	*n*	Mean Body Mass (g)	Unadjusted RMR (mg O_2 h^{-1})	Adjusted RMR (mg O_2 h^{-1})	Critical Oxygen Tension (mm Hg)	Slope in Conformation Region
0	11	3.17 ± 0.232	0.81 ± 0.038	0.78 ± 0.025	56.98 ± 6.92	0.0071 ± 0.0024
2	7	3.38 ± 0.258	1.04 ± 0.017	0.97 ± 0.027	51.49 ± 5.85	0.0079 ± 0.0039
15	8	2.95 ± 0.438	0.75 ± 0.048	0.78 ± 0.029	53.68 ± 4.92	0.0072 ± 0.0021
30	10	3.57 ± 0.402	0.88 ± 0.033	0.83 ± 0.020	52.16 ± 5.10	0.0058 ± 0.0018
40	9	2.19 ± 0.337	0.76 ± 0.050	0.96 ± 0.030	52.14 ± 2.70	0.0079 ± 0.0017
50	18	3.69 ± 0.322	0.94 ± 0.031	0.87 ± 0.020	61.81 ± 4.89	0.0065 ± 0.0011
60	8	3.42 ± 0.491	0.83 ± 0.057	0.80 ± 0.030	63.41 ± 6.06	0.0033 ± 0.0008
70	12	2.70 ± 0.389	0.65 ± 0.050	0.73 ± 0.025	66.31 ± 5.57	0.0041 ± 0.0006
80	12	2.98 ± 0.437	0.67 ± 0.047	0.70 ± 0.025	79.53 ± 5.61	0.0033 ± 0.0004
90	8	3.52 ± 0.362	0.73 ± 0.040	0.68 ± 0.029	74.94 ± 8.00	0.0034 ± 0.0004
100	8	3.21 ± 0.460	0.63 ± 0.032	0.64 ± 0.030	73.93 ± 8.78	0.0034 ± 0.0005

Figure 2-1. Mean adjusted routine metabolic rates (RMR) over a range of salinities in *Cyprinodon variegatus* (metabolic rates were mass-adjusted using an analysis of covariance; bars indicate ± se; numerical values above the points in the figure indicate sample size at each salinity).

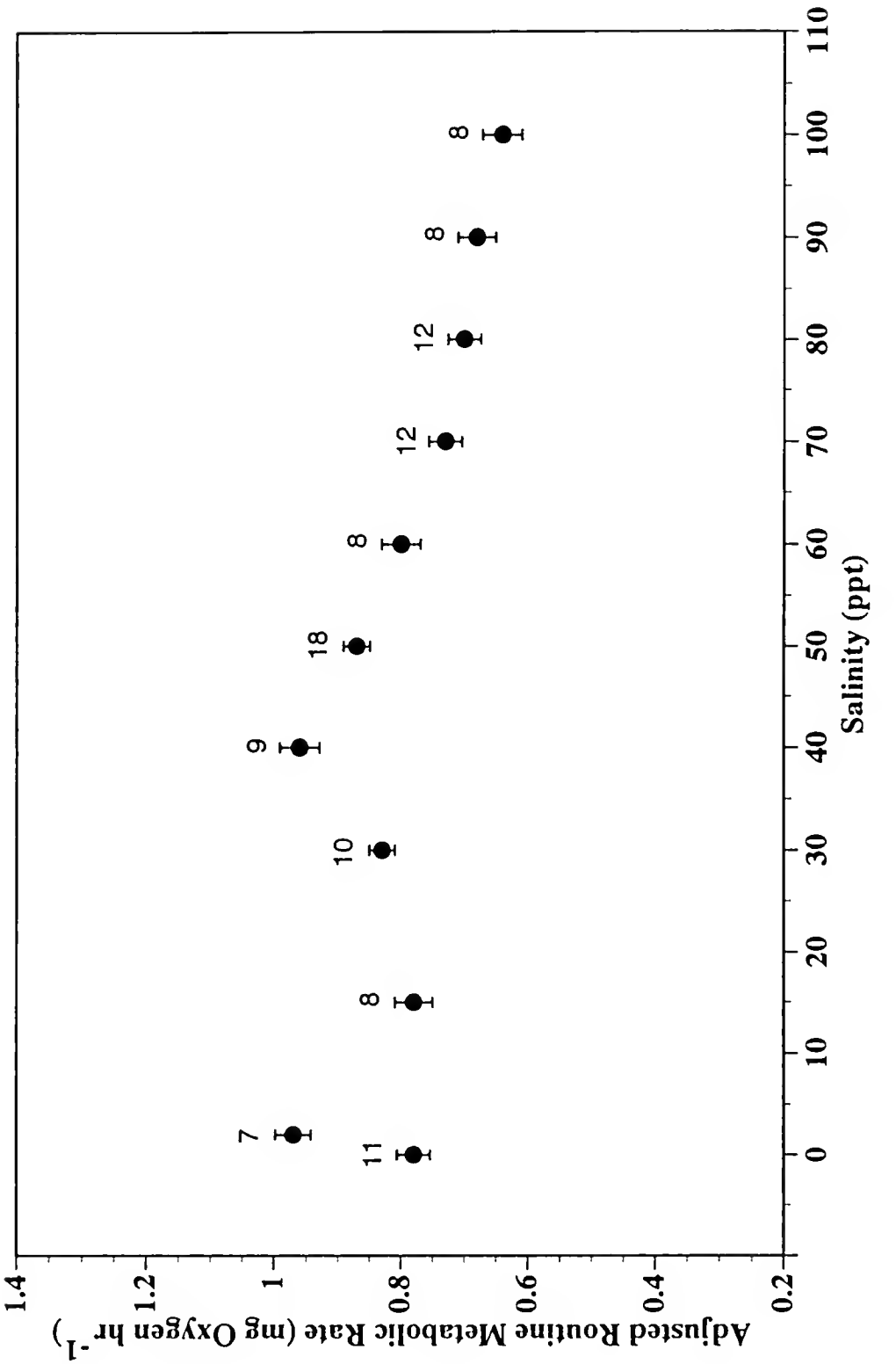

Adjusted Routine Metabolic Rate (mg Oxygen hr-1)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
20
30
40
50
60
70
80
90
100
110
Salinity (ppt)
7
11
8
10
9
18
8
12
12
8
8

Figure 2-2. Relationship between mean adjusted routine metabolic rates (RMR) and mean plasma osmolality over a range of salinities in *Cyprinodon variegatus* (metabolic rates were mass-adjusted using an analysis of covariance; bars indicate ± se; plasma osmolality data from Nordlie, 1985).

Adjusted Routine Metabolic Rate
(mg Oxygen/hr)
Plasma Osmolality (mOsm/kg)
Salinity (ppt)
Routine Metabolic Rate
Plasma Osmolality
Log MR = - 0.296 + 0.548(Log W) - 0.001(S)
(P < 0.0001)

Critical Oxygen Tension

Measurements of oxygen consumption (mg O_2 h^{-1}) for each fish at all time intervals were mass-adjusted to the mean mass of all individuals used in this study. These mass-adjusted values were then used to calculate the P_c for each fish. Mean values of P_c were organized by salinity group and are given in Table 2-1. Plots showing the relationship between P_c and ambient salinity and between P_c, RMR, and ambient salinity are shown in Figures 2-3 and 2-4, respectively.

Mean P_c values in the range of ambient salinities from 0 ppt to 40 ppt were not significantly different from one another ($p = 0.95$), similar to the pattern exhibited by the RMR data. Mean P_c values increased at salinities greater than 40 ppt, with the highest levels recorded at salinities 80 ppt and higher. P_c values ranged from a low of 51.49 mm Hg at a salinity of 2 ppt, to a high of 79.50 mm Hg at 80 ppt, representing a 45% increase. The rise in mean P_c values corresponds well with a decreased ability to regulate plasma osmolality, again similar to the RMR pattern (Figure 2-5; plasma osmolality data are from Nordlie, 1985).

Below the P_c, metabolism depends on the oxygen tension and decreases as the PO_2 decreases. Mathematically, the slope of the resulting line in this conformation region depends on three factors: the P_c, the RMR at the P_c, and the lethal PO_2. A comparison of the slopes in the conformation region reveals that despite the changes noted above in RMR and P_c values, the PO_2 at which the fish can no longer survive (under experimental conditions) is essentially equivalent for all salinities tested. This is reflected in the increasingly shallow slopes seen at salinities greater than 50 ppt (Table 2-1).

Field Measurements

The field measurements of oxygen concentration, salinity, and temperature revealed very high variability of these physicochemical parameters over the course of the sampling

Figure 2-3. Mean critical oxygen tension (P_C) measurements over a range of salinities in *Cyprinodon variegatus* (bars indicate ± se; numerical values above the points in the figure indicate sample size at each salinity).

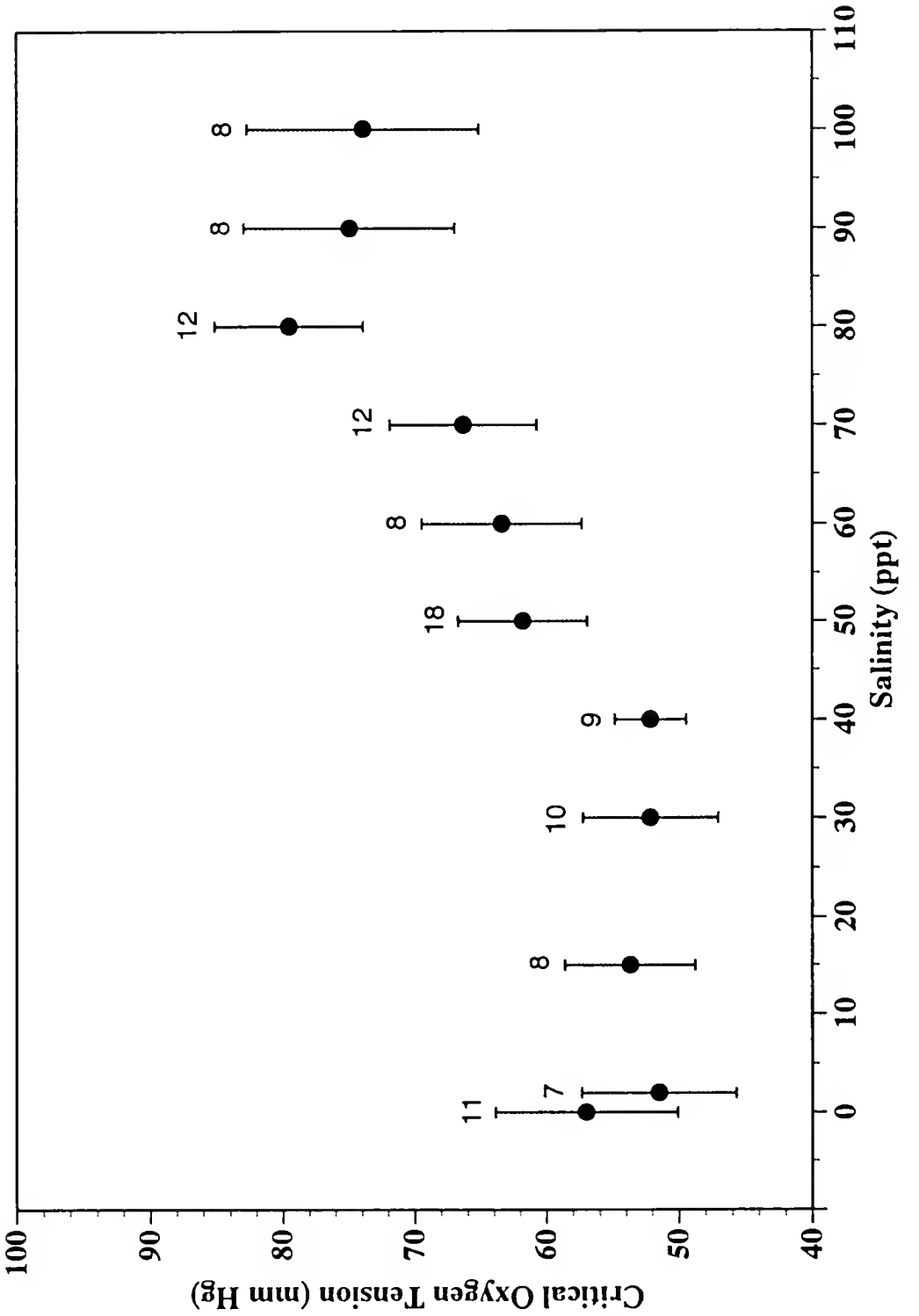

Critical Oxygen Tension (mm Hg)
100
90
80
70
60
50
40
0
10
20
30
40
50
60
70
80
90
100
110
Salinity (ppt)
11
7
8
10
9
18
8
12
12
8
8

Figure 2-4. Relationship between mean adjusted routine metabolic rates (RMR) and critical oxygen tensions (P_C) over a range of salinities in *Cyprinodon variegatus* (metabolic rates were mass-adjusted using an analysis of covariance; bars indicate ± se).

Adjusted Routine Metabolic Rate
(mg Oxygen/hr)
Critical Oxygen Tension (mm Hg)
Salinity (ppt)
Routine Metabolic Rate
Critical Oxygen Tension
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
100
90
80
70
60
50
40
0
10
20
30
40
50
60
70
80
90
100
110

Figure 2-5. Relationship between mean critical oxygen tensions (P_c) and mean plasma osmolality over a range of salinities in *Cyprinodon variegatus* (bars indicate ± se; plasma osmolality data from Nordlie, 1985).

Critical Oxygen Tension (mm Hg)
Plasma Osmolality (mOsm/kg)
Salinity (ppt)
Critical Oxygen Tension
Plasma Osmolality
100
90
80
70
60
50
40
700
650
600
550
500
450
400
350
300
0
10
20
30
40
50
60
70
80
90
100
110

period (Table 2-2). For all sites combined, ranges of dissolved oxygen, salinity, and temperature over the course of the year were 0.6 - 10.8 mg L^{-1}, 1.0 - 29.0 ppt, and 9.0 - 38.0 ^{o}C, respectively. Even higher salinities (> 40 ppt) were encountered occasionally in the salt marsh outside the sampling period. Other areas of the salt marsh probably encounter even greater extremes of these variables. C*yprinodon variegatus* was seen on all dates when physicochemical measurements were made.

In addition to high overall variability, there were large differences among some sites in close proximity to one another. For example, mean oxygen concentration measurements taken during the Fall at sites one, two, and three were 2.94 mg L^{-1}, 3.85 mg L^{-1}, and 5.1 mg L^{-1}, respectively. All three physicochemical parameters also strongly varied temporally among sampling dates. These data provide good evidence that the Cedar Key salt marsh is an extremely variable habitat with respect to these physicochemical parameters.

Discussion

The family Cyprinodontidae is a diverse group of fishes with many species that tolerate extreme environmental conditions (Lowe et al., 1967; Lotan and Skadhauge, 1972; Naiman et al., 1976; Stuenkel and Hillyard, 1981; Chung, 1982). *Cyprinodon variegatus* is perhaps the most physiologically tolerant member of the family. It has been called "the toughest fish in North America" (Gunter, 1967) due to its extreme tolerance of severe abiotic conditions. The species is found in waters ranging in salinity from freshwater (Ager, 1971) to 142 ppt (Simpson and Gunter, 1956), and can reproduce in waters as high as 100 ppt (Martin, 1972). They are known to tolerate temperatures ranging from about 1 ^{o}C (Berry, 1987), to temperatures greater than 41 ^{o}C (Strawn and Dunn, 1967), and to tolerate near anoxic conditions (Odum and Caldwell, 1955). Thus, this species is an exceedingly useful experimental subject for examining how teleosts respond to harsh environmental conditions.

Table 2-2. Measurements of oxygen concentration (mg L^{-1}), salinity (ppt), and temperature (^{o}C) taken at four sites in the Cedar Key area from June 1990 through June 1991 (see text for details on location of sites). Values are given as means, se (in parentheses), sample size.

		Oxygen (mg L^{-1})				Salinity (ppt)				Temperature (^{o}C)			
Location	Depth	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
Site 1	Bottom	2.18 (0.28) n = 5	3.15 (0.54) n = 8	2.63 (0.42) n = 4	4.24 (0.97) n = 5	13.9 (1.05) n = 5	16.63 (1.45) n = 8	12.75 (2.62) n = 4	11.3 (1.69) n = 5	25.2 (0.37) n = 5	30.50 (1.03) n = 8	21.75 (2.39) n = 4	14.90 (1.35) n = 5
	Surface	3.08 (0.38) n = 5	3.72 (0.60) n = 6	3.25 (0.51) n = 4	5.16 (1.36) n = 5	11.00 (1.04) n = 5	15.92 (2.88) n = 6	13.75 (2.44) n = 4	10.60 (0.80) n = 5	25.60 (1.37) n = 5	31.83 (1.49) n = 6	21.63 (2.93) n = 4	15.00 (1.18) n = 5
Site 2	Bottom	1.98 (0.47) n = 5	2.37 (0.70) n = 8	2.65 (0.63) n = 4	6.78 (1.33) n = 5	12.30 (2.65) n = 5	13.56 (2.31) n = 8	12.50 (2.73) n = 4	7.00 (0.91) n = 5	24.8 (0.84) n = 5	29.36 (0.69) n = 8	20.00 (2.81) n = 4	14.40 (1.54) n = 5
	Surface	3.35 (0.36) n = 5	4.83 (0.56) n = 6	5.05 (0.89) n = 4	8.56 (0.66) n = 5	6.2 (1.47) n = 5	11.08 (3.29) n = 6	7.50 (2.60) n = 4	4.20 (0.72) n = 5	24.70 (0.97) n = 5	30.42 (0.84) n = 6	20.13 (3.18) n = 4	14.60 (1.63) n = 5
Site 3	Bottom	3.83 (0.43) n = 5	3.46 (1.02) n = 5	5.08 (0.96) n = 4	8.24 (0.48) n = 5	6.80 (1.97) n = 5	7.20 (2.96) n = 5	7.88 (3.18) n = 4	4.60 (0.78) n = 5	24.90 (0.78) n = 5	30.40 (1.29) n = 5	21.38 (3.18) n = 4	16.60 (1.29) n = 5
	Surface	3.65 (0.25) n = 3	5.03 (0.73) n = 3	5.15 (0.15) n = 2	--- --- ---	4.50 (0.50) n = 3	5.33 (1.92) n = 3	3.00 (2.00) n = 2	--- --- ---	25.30 (1.33) n = 3	31.17 (1.59) n = 3	25.50 (0.5) n = 2	--- --- ---
Site 4	Middle	5.10 (0.68) n = 5	5.37 (0.85) n = 7	4.35 (0.60) n = 4	8.06 (0.98) n = 5	17.75 (3.13) n = 5	27.36 (0.38) n = 7	23.88 (0.52) n = 4	15.50 (2.42) n = 5	27.0 (0.51) n = 5	29.71 (0.38) n = 7	22.50 (2.76) n = 4	15.10 (1.36) n = 5

Field measurements indicated that the Cedar Key salt marsh varies tremendously, both spatially and temporally, in dissolved oxygen, temperature, and salinity, thus exposing organisms living within the salt marsh to extreme environmental conditions. *Cyprinodon variegatus* tolerates these conditions very well. Salinities between 0 ppt and 40 ppt have little effect on the energetics of *C. variegatus* as measured by RMR and P_C. This species fits into the Type I metabolic response pattern (species exhibiting no significant change in metabolic rate over a wide range of environmental salinities) as categorized by both Nordlie (1978) and Morgan and Iwama (1991). A similar pattern characterizes the related salt marsh resident, *Adinia xenica* (D.C. Haney, unpublished data), in which RMR was more variable, but relatively constant over salinities from 0 ppt to 50 ppt (Figure 2-6).

Cyprinodon variegatus exhibits a decline in RMR only at salinities exceeding 40 ppt. This result was somewhat unexpected, but is nearly identical to the pattern found by Nordlie et al., (1991) in a study that also examined metabolism of *C. variegatus* over a wide range of ambient salinities. Nordlie et al., (1991) concluded that the depression in metabolism at high salinities is probably related to permeability changes of the gill membrane and/or integument. The point at which metabolism is reduced corresponds well with a diminished ability of *C. variegatus* to osmoregulate efficiently. If osmotic permeability of the gills is reduced at high salinities to help offset ionic influx and osmotic efflux, the potential for oxygen uptake may be reduced as well (Kristensen and Skadhauge, 1974; Skadhauge, 1974; Davenport and Sayer, 1993). Evidence for this hypothesis comes from several recent studies. In the first of these, Kultz and Onken (1993) found that overall *in vitro* permeability of the opercular membrane of the cichlid *Oreochromis mossambicus* was reduced in hypersaline media, with a simultaneous reduction in passive ion fluxes. More direct evidence comes from studies by Bindon et al., (1994a) and Bindon et al., (1994b), on the rainbow trout, *Oncorhynchus mykiss*. In these studies the authors demonstrated that impairment of respiratory gas transfer coincided with chloride cell proliferation induced by an osmoregulatory challenge.

Figure 2-6. Relationship between mean adjusted routine metabolic rates (RMR) and mean plasma osmolality over a range of salinities in *Adinia xenica* (metabolic rates were mass-adjusted using an analysis of covariance; bars indicate ± se; numerical values above the points in the figure indicate sample size at each salinity).

Adjusted Routine Metabolic Rate
(Mg Oxygen/hr)
Plasma Osmolality (mOsm/Kg)
Salinity (ppt)
Routine Metabolic Rate
Plasma Osmotic Concentration
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
600
550
500
450
400
350
300
0
10
20
30
40
50
60
8
6
13
5
8

Changes in activity may also account for a depression in metabolism. In a study on the milkfish, *Chanos chanos*, Swanson (1991) showed that a depression in metabolism at an elevated salinity (55 ppt) was highly correlated with a reduction in activity. This may have been a factor in this study although the method used here for metabolic determinations minimized activity of fish at all salinities.

The main focus of this study was determination of P_c over a wide salinity range, as no other study has investigated to any extent the influence of salinity on this parameter. Critical oxygen tension can be affected by many factors. These include both physical and biotic parameters such as temperature, salinity, size and activity, reproductive and nutritional state, and experimental parameters, such as rate of oxygen depletion or manipulation and disturbance of fish during measurements (McMahon, 1988).

Like other vertebrates, *C. variegatus* is a good oxygen regulator over a wide range of oxygen tensions. Critical oxygen tension in *C. variegatus* was unaffected by changes in salinity between 0 ppt and 40 ppt, similar to the result for RMR. However, above 40 ppt, P_c increased with further increases in salinity. This seems reasonable, as fish might be expected to encounter difficulties obtaining sufficient oxygen at elevated salinities.

Unfortunately, it is difficult to compare my results with those from other groups of fishes. While the P_c has been measured for a number of freshwater, marine, and intertidal fishes, only a few authors have considered the effects of salinity in their analyses. Job (1969) examined oxygen consumption of *Tilapia mossambica* (= *O. mossambicus*) at 0.4 ppt, 12.5 ppt, and 30.5 ppt, and found no effect of salinity on the P_c of small (5 g) fish at either 15 ^{o}C, 30 ^{o}C, or 40 ^{o}C (P_c approximately 50 mm Hg). Salinity did affect P_c on larger (80 g) individuals at 15 ^{o}C, where the P_c doubled from approximately 50 mm Hg to 100 mm Hg in fish acclimated to 0.4 ppt versus 12.5 ppt or 30.5 ppt. However, P_c calculations made by Job (1969) were extremely rough approximations, and are difficult to compare with values in this study.

Subrahmanyam (1980) examined the influence of oxygen tension on the metabolic rate of several salt marsh fishes including *C. variegatus*, with all measurements made at 25 ^{o}C and salinities of 17-21 ppt. All species tested in Subrahmanyam (1980) (*C. variegatus, Poecilia latipinna, Lagodon rhomboides, Leiostomus xanthurus, Fundulus grandis, and F. similis*) were oxygen conformers at oxygen tensions of 85 mm Hg and lower, a somewhat different response than seen in this study. This difference may be due to the small sample sizes, and relatively few measurements of oxygen consumption made by Subrahmanyam (1980). In a study on the golden mullet, *Liza aurata*, Shusmin (1989), measured the "oxygen threshold" at salinities ranging from freshwater to 50 ppt. He showed that the oxygen threshold was relatively constant at all salinities between freshwater and 40 ppt, with a large increase at 50 ppt. *Cyprinodon variegatus* did not show this type of response, as the lethal endpoint does not appear to increase at higher salinities in this species.

Values of the P_c in this study compare fairly well with P_c measurements for other groups of fishes. Intertidal marine species generally have lower P_c values than *C. variegatus*, ranging from 20 to 26 mm Hg in *Paraclinus intergripinnis* (Congleton, 1980) to 30 to 40 mm Hg in *Gobius cobitus* (Bridges, 1988) and *Helcogramma medium* (Innes and Wells, 1985; Pelster et al., 1988; Quinn and Schneider, 1991). A number of African cichlids (e.g., *Oreochromis niloticus, Cichlasoma urophthalamus, Eretmodus cyanosticus, Dimidiochromis compressiceps*) have either slightly lower, or roughly equivalent P_c values to the approximate value (55 mm Hg) displayed by *C. variegatus* in this study at most salinities (Ross and Ross, 1983; Becker and Fishelson, 1986; Palacios and Ross, 1986; Verheyen et al., 1994). Donnelly and Torres (1988) found P_c values ranging from 25 to 50 mm Hg for a number of midwater fishes from the eastern Gulf of Mexico, again values near, or slightly lower than those of *C. variegatus*. Thus, *C. variegatus* has P_c values that are fairly close to those of ecologically and evolutionarily diverse teleosts.

The variations in RMR and P_c as a function of environmental salinity observed in this study suggest that *C. variegatus* responds to high salinities by reducing energy expenditures. Observations by myself and others indicate that hypersaline conditions are encountered infrequently, and likely last for short (days) periods of time. Available evidence suggests that high salinities are metabolically expensive, whether due to an influence on activity, osmoregulation, or other physiological and/or behavioral processes. Low oxygen conditions also commonly occur with high salinities. Decreased metabolism in conjunction with increased P_c reduces energetic expenditures dramatically at elevated salinities. These responses effectively increase the time *C. variegatus* can tolerate adverse conditions, albeit at a cost of a reduction in energetic processes. However, this is an appropriate response to harsh conditions that appear at variable and unpredictable time intervals, but that are present for only short periods of time.

The type of metabolic response to salinity in *C. variegatus* fits the concept of "scope for survival" as described by Hochachka (1990). This is a pattern of metabolic response to environmental stressors characterized by a depression in metabolism, sometimes below maintenance levels. The advantage of such a reduction in energy expenditure is essentially the slowing of biological time, enabling survival despite the temporarily imposed physiological stressor.

Cyprinodon variegatus is an extremely successful euryhaline fish that can survive a large range of salinities, with typically encountered salinities having little effect on normal metabolism or on the ability of individuals to osmoregulate efficiently. Fish accomodate extremely high salinities by reducing energetic expenditures. Such a response increases the amount of time sheepshead minnows can survive hypersaline conditions, enabling them to "wait out" the difficult conditions.

CHAPTER 3
INFLUENCE OF SIMULATED TIDAL CHANGES IN AMBIENT SALINITY ON ROUTINE METABOLIC RATE IN *CYPRINODON VARIEGATUS*

Introduction

Salt marshes often undergo large and rapid salinity fluctuations, a condition that may significantly affect the distribution and abundance of organisms within these habitats. Changes in salinity may dramatically influence the energetics of individual fish. Information on the metabolic costs associated with salinity fluctuations may be useful in explaining or predicting distribution patterns of some coastal fish species.

Energetic patterns of fishes have been examined relative to a number of abiotic factors, most notably temperature and oxygen (e.g., Wells, 1935; Fry, 1957; Beamish, 1964; Brett and Groves, 1979; Stuenkel and Hillyard, 1981; Johnston and Battram, 1993). Comparatively fewer studies have been considered the influence of salinity on metabolic patterns of fishes (e.g., Kinne, 1966; Madan Mohon Rao, 1974; Nordlie et al., 1991; Swanson, 1991). In these studies, various metabolic responses to salinity have been reported. Euryhaline fish exhibit one of the few consistent patterns, namely that metabolism remains relatively unaffected over the range of salinities normally encountered (Nordlie, 1978; Morgan and Iwama, 1991).

Most studies that have examined the influence of salinity on fish energetics have employed measurements from fish maintained at constant salinities. Data from these experiments are very useful for understanding the overall metabolic patterns exhibited by the species tested, as fish were usually fully acclimated to each experimental salinity prior to testing. However, these experiments provide less information about more ecologically

relevant responses, as fish in their native habitats are often subject to rapid and dramatic fluctuations in salinity.

Studies of the influence of salinity fluctuations on metabolism of fishes have usually focused on species that generally experience maximal fluctuations in salinity only between freshwater (0 ppt) and seawater (35 ppt) (e.g., Davenport and Vahl, 1979; Von Oertzen, 1984; Moser and Gerry, 1989; Shusmin, 1989; Moser and Miller, 1994). However, some salt marsh teleosts regularly encounter salinities outside this range. This study examined the influence of salinity fluxes on routine metabolic rate (RMR) of the salt marsh teleost, *Cyprinodon variegatus*, a species that regularly encounters salinities greater than 35 ppt.

Fish used in this study were fully acclimated to a series of salinities ranging from 0 ppt to 60 ppt, followed by exposure to a simulated tidal change in salinity. The magnitude, rate, and direction of salinity changes may be important determinants of how salinity fluctuations affect metabolism. In this study, the direction of the salinity change in conjunction with the ambient (acclimation) salinity was the primary focus. The magnitude of the salinity changes were selected to simulate extremes known to occur in the habitat from which fish were collected, and the rate was chosen to simulate a normal diurnal tidal cycle. I predicted that *C. variegatus* would show minimal changes in RMR when the salinity was changed within the range commonly encountered by the species. Salinity changes outside this range were hypothesized to lead to depressions in metabolism.

Methods

Collections of fish, transportation back to the laboratory, and general laboratory procedures were performed as described previously. Fish were first sequentially acclimated to a series of salinities ranging from 0 ppt to 60 ppt (0 to 1715 mOsm kg^{-1}). However, unlike the previous experiments, these procedures were designed to measure RMR before

and following simulated tidal changes in salinity. Initial salinities (acclimation) and salinities following the simulated tidal change (final) are shown in Table 3-1.

Metabolic determinations and changes in salinity were carried out in a flow-through respirometer with an effective volume of 0.875 L (Figure 3-1). The respirometer was immersed in a thermoregulated reservoir during metabolic trials that served to maintain a constant temperature within the respirometer. A submersible pump maintained within a second thermoregulated reservoir (38 L) was connected to the inlet of the respirometer. This reservoir was vigorously aerated and was used to supply water to the respirometer. The respirometer outlet emptied back into this main reservoir. A third reservoir (19 L) serving as a salinity source was connected to the main reservoir via a peristaltic pump. Due to limitations of the available equipment, the system was closed during oxygen partial pressure (PO_2) determinations. Two ports within the respirometer were used to sample water for determination of PO_2. A 10 ml plastic syringe filled with water at the experimental salinity was inserted into the first port (inlet side), while an empty 10 ml syringe was fitted into the second port (outlet side). As a 1 ml water sample was drawn into the empty syringe, an equal volume of water was injected from the filled syringe into the respirometer. Determinations of PO_2 were made with a Radiometer® oxygen electrode connected to a Radiometer® PHM171 acid-base analyzer.

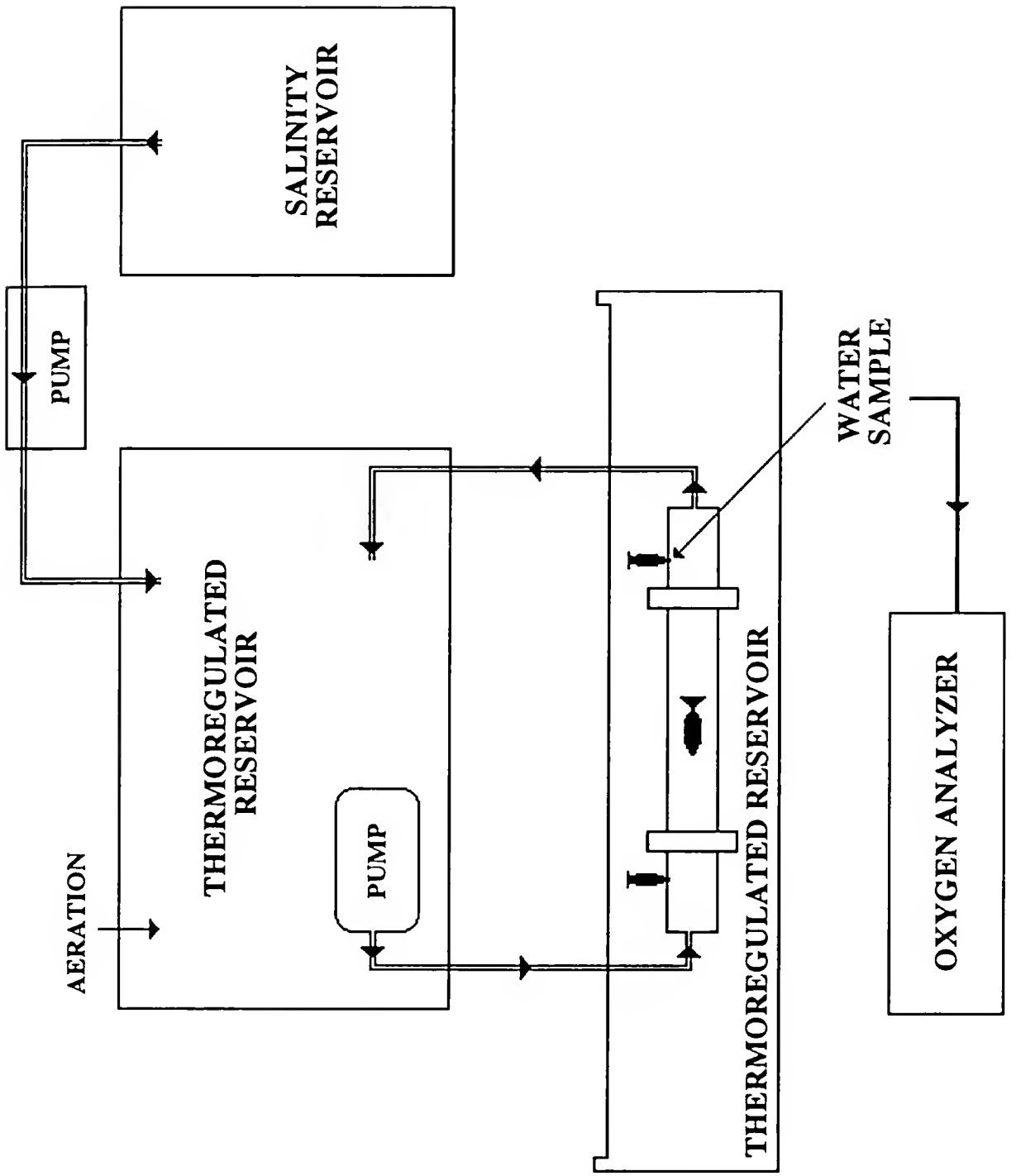
To begin a metabolic trial, the main reservoir and respirometer were filled with water at the acclimation salinity. Fish were placed into the respirometer 12 h prior to the beginning of a metabolic determination. This allowed fish sufficient time to adjust to the experimental setup. The respirometer was then sealed and immersed in the thermoregulation reservoir, and the submersible pump was turned on at a flow rate of 1 L min^{-1}. The following morning, the pump was turned off, the system was closed, and PO_2 measurements begun. Measurements of the rate of reduction in PO_2 were made at 0.5 to 1.0 h intervals, and continued until fish had depleted the oxygen level to approximately 100 mm Hg (generally 4 to 6 h). Following the final PO_2 determination, the system was

Table 3-1. Acclimation and final salinities used in simulated tidal change study.

Acclimation Salinity	Final Salinity
0 ppt	0 ppt (control) 20 ppt
2 ppt	2 ppt (control) 20 ppt 30 ppt
10 ppt	10 ppt (control) 30 ppt
20 ppt	0 ppt 2 ppt 20 ppt (control) 40 ppt 50 ppt
30 ppt	2 ppt 10 ppt 30 ppt (control) 50 ppt
40 ppt	20 ppt 40 ppt (control) 60 ppt
50 ppt	20 ppt 30 ppt 50 ppt (control)
60 ppt	40 ppt 60 ppt (control)

reopened, and the submersible pump turned on. At this time, either well water (0 ppt) or a saline solution of variable concentration was placed into the salinity reservoir. The salt concentration in this reservoir was determined so that its addition to the main reservoir would change the salinity of the main reservoir and respirometer to the desired final salinity.

Figure 3-1. Schematic diagram of respirometry apparatus used for routine metabolism experiments. See text for detailed description of system.


SALINITY RESERVOIR
PUMP
THERMOREGULATED RESERVOIR
AERATION
PUMP
WATER SAMPLE
THERMOREGULATED RESERVOIR
OXYGEN ANALYZER

The volumes of both reservoirs and the rate of pumping by the peristaltic pump were adjusted so that the salinity would change at a uniform rate over a 6 h period. The peristaltic pump was plugged into an automatic timer so that salinity changes took place between 1900 h to 0200 h.

On the second morning the system was re-closed and PO_2 measurements were made at the new (final) salinity. PO_2 measurements at the final salinity were thus made 6-12 h following completion of the salinity fluctuation, a period sufficiently long to be past the stressful transition period (Von Oertzen, 1984). Following the final PO_2 determination, the fish was removed from the respirometer, damp-dried and weighed to the nearest 0.01 g. All metabolic determinations were made between 0700 h and 1500 h, and fish were not re-used in other metabolic trials. Fish were thus held within the respirometer for a total of 40 to 44 h (including adjustment period).

Food was withheld from experimental aquaria for 24 h prior to beginning a metabolic reading to ensure that fish were post-absorptive at the time of testing. Fish were not fed while in the respirometer. The entire metabolic apparatus was located in a small, semi-darkened room in which there was no other activity, with determination of metabolic rates performed as previously described.

Because a metabolic determination for the two salinity treatments was performed on the same individual, it was not necessary to mass-correct values in order to perform the statistical analyses. However, to compare data obtained in this study more easily with published literature, all RMR measurements were mass-adjusted to the value of the average mass (2.94 g) of all individuals used in this study. Adjusted RMR values were calculated as previously described. A repeated measures analysis of variance was used to compare adjusted RMR's between acclimation and final salinities. Statistical analyses follow procedures outlined in Winer et al. (1991) and Sokal and Rohlf (1995). All analyses of variance were one way tests using the Tukey-Kramer post hoc comparison ($p = 0.05$).

Results

Comparison of RMR's at acclimation and final salinities revealed some interesting patterns (Table 3-2). There were no significant differences ($p > 0.05$) in RMR between acclimation and final salinities in any control trials ($n = 4$ each). Similarly, when both acclimation and final salinities (2 ppt, 10 ppt, 20 ppt, 30 ppt, and 40 ppt) were in a range that is typically encountered by *C. variegatus*, there was a significant change in RMR in only one trial. Typical salinities in the wild range from 2-5 ppt through 30-35 ppt, with hypersaline conditions (35-40 ppt) occurring much more frequently than salinities near 0 ppt (pers. obs.). When the acclimation salinity is high (50 ppt and 60 ppt), all groups exhibited significant elevation of RMR in the lower, more typical, final salinity. The same general pattern was seen when the final salinity was high, where fish in two of three groups had depressed RMR at the highest salinities. RMR was depressed when either the acclimation or final salinity was 0 ppt. The direction of salinity change strongly influenced the metabolic response. When salinity was increased over the course of the trial (Figure 3-2), fish were only affected metabolically at the very highest and lowest salinities, where metabolism was depressed. When salinity was decreased over the course of the trial (Figure 3-3), fish again showed metabolic depression at extreme salinities, but, overall, more groups exhibited changes in metabolism with changes in salinity than groups in which salinity was increased.

Discussion

My results demonstrate that *C. variegatus* maintains a very stable metabolism when exposed to typical salinity fluctuations seen in the salt marsh habitat at Cedar Key, Florida. The experimental procedure resulted in no mortalities during or immediately following any salinity trial. Metabolism was generally unaffected in salinity trials where both acclimation and final salinities were in the range typically encountered by *C. variegatus* at Cedar Key. It

Table 3-2. Mean routine metabolism (mg O_2 h^{-1}) before (acclimation salinity) and following (final salinity) a simulated tidal change. Values are given as means ± se. Groups exhibiting a significant change in metabolism are indicated with an asterisk.

Acclimation Salinity (ppt)	Acclimation RMR (mg O_2 h^{-1})	Final Salinity (ppt)	Final RMR (mg O_2 h^{-1})	P value	Response
0 ppt	0.466 ± 0.07	20 ppt (n = 5)	0.584 ± 0.09	0.04 *	Increase
2 ppt	1.242 ± 0.18	20 ppt (n = 5)	1.244 ± 0.23	0.99	No Change
	1.024 ± 0.13	30 ppt (n = 5)	0.978 ± 0.06	0.69	No Change
10 ppt	1.084 ± 0.20	30 ppt (n = 5)	1.082 ± 0.19	0.97	No Change
20 ppt	1.348 ± 0.14	0 ppt (n = 5)	0.906 ± 0.09	0.01 *	Decrease
	0.886 ± 0.03	2 ppt (n = 5)	0.478 ± 0.07	0.01 *	Decrease
	0.948 ± 0.08	40 ppt (n = 6)	0.785 ± 0.17	0.33	No Change
	1.025 ± 0.11	50 ppt (n = 6)	0.745 ± 0.07	0.01 *	Decrease
30 ppt	1.028 ± 0.23	2 ppt (n = 5)	0.854 ± 0.10	0.33	No Change
	0.628 ± 0.07	10 ppt (n = 5)	0.688 ± 0.11	0.34	No Change
	0.728 ± 0.07	50 ppt (n = 6)	0.728 ± 0.12	1.00	No Change
40 ppt	0.704 ± 0.12	20 ppt (n = 5)	0.662 ± 0.09	0.75	No Change
	0.626 ± 0.06	60 ppt (n = 5)	0.476 ± 0.03	0.03 *	Decrease
50 ppt	0.620 ± 0.04	20 ppt (n = 5)	0.904 ± 0.11	0.05 *	Increase
	0.850 ± 0.12	30 ppt (n = 5)	1.076 ± 0.14	0.05 *	Increase
60 ppt	0.452 ± 0.07	40 ppt (n = 5)	0.578 ± 0.05	0.01 *	Increase

was only when the acclimation or final salinities were outside the range normally encountered (i.e., 0 ppt, 50 ppt, and 60 ppt) that changes in metabolism were found. In these cases metabolism was depressed at each extreme salinity relative to the measurements made at the more typical salinity.

Figure 3-2. Results of metabolic trials where salinity was increased over the course of the trial. Bars represent groups listed in Table 3-2 for which final salinity was greater than initial salinity. The height of each bar signifies the magnitude of the salinity change for each metabolic trial and the asterisk indicates at which of the salinities (for each metabolic trial) the routine metabolic rate (RMR) was highest. The *x* axis has no scale and serves only to visually separate groups.

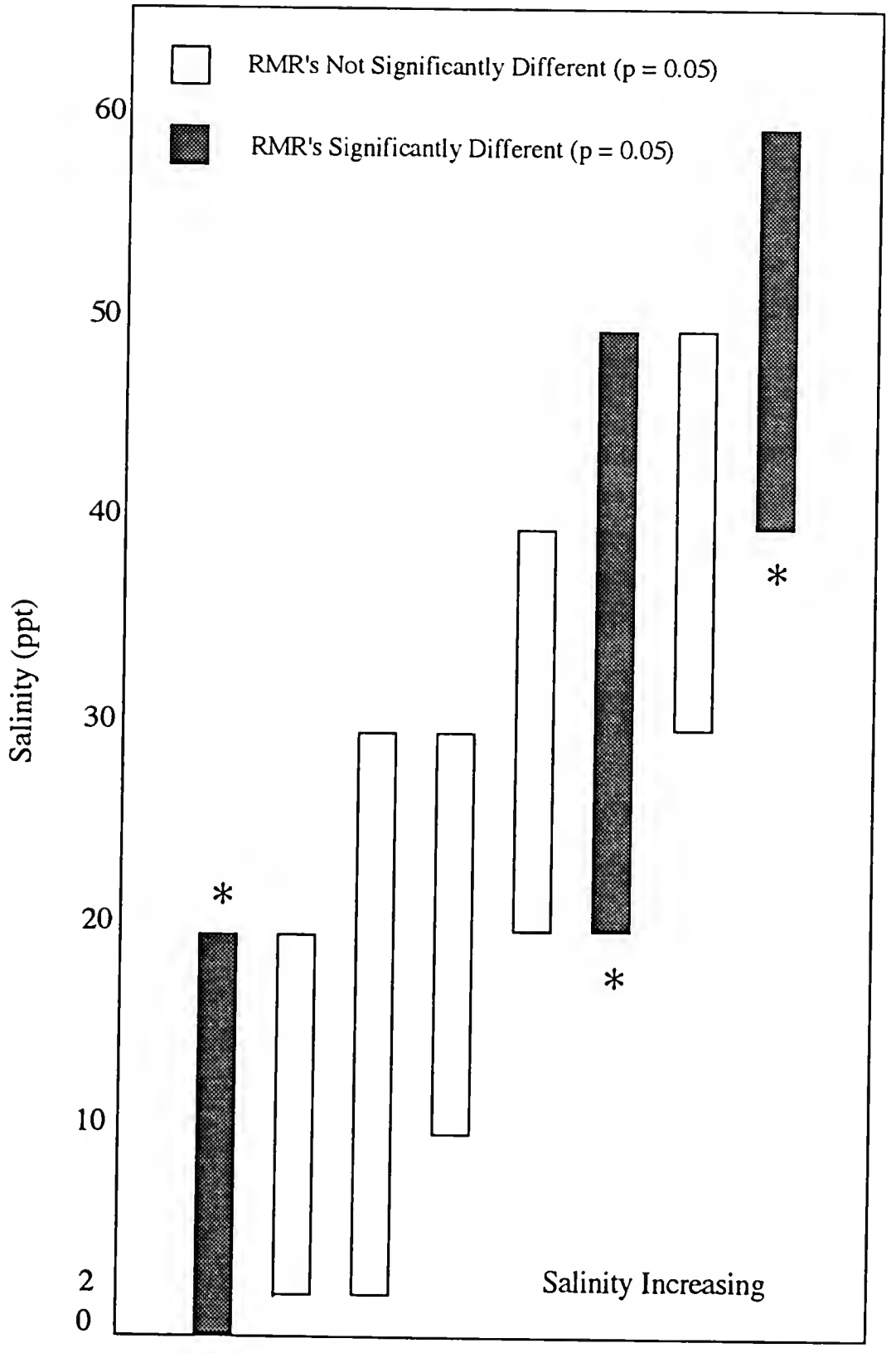
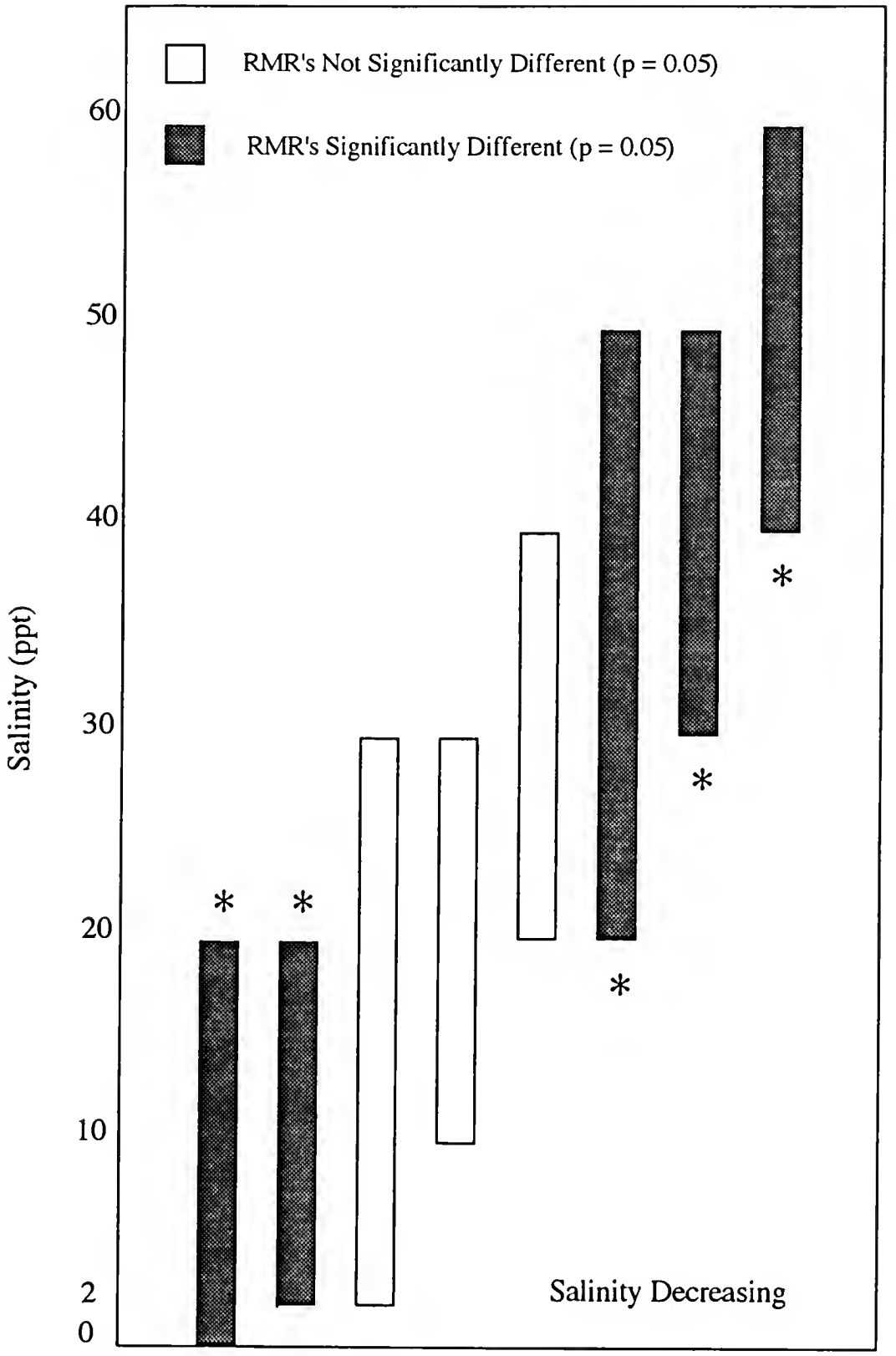

RMR's Not Significantly Different (p = 0.05)
RMR's Significantly Different (p = 0.05)
60
50
40
30
20
10
2
0
Salinity (ppt)
Salinity Increasing
*
*
*

Figure 3-3. Results of metabolic trials where salinity was decreased over the course of the trial. Bars represent groups listed in Table 3-2 for which final salinity was less than initial salinity. The height of each bar signifies the magnitude of the salinity change for each metabolic trial and the asterisk indicates at which of the salinities (for each metabolic trial) the routine metabolic rate (RMR) was highest. The *x* axis has no scale and serves only to visually separate groups.


RMR's Not Significantly Different (p = 0.05)
RMR's Significantly Different (p = 0.05)
60
50
40
30
20
10
2
0
Salinity (ppt)
Salinity Decreasing
*
*
*
*
*

These results allow for limited comparison because similar studies have examined the influence of salinity over a much narrower range than examined in this study. The best comparison is with a study by Wakeman and Wohlschlag (1983) on the red drum, *Sciaenops ocellatus*. In their study, test animals were acclimated to a series of salinities between 1 ppt and 50 ppt, and then abruptly transferred to salinities either 10 ppt higher or lower than the acclimation salinity. Although Wakeman and Wohlschlag (1983) did not specifically compare metabolic rates following the transfer with those taken at the acclimation salinity, there were trends similar to results of this study. Fluctuations in salinity over a moderate range caused little or no change in metabolism of *S. ocellatus* on determinations made 12 to 24 h following the transfer. Only when either the acclimation or final salinity was 50 ppt were changes in metabolism obvious, with metabolic rates elevated in 50 ppt compared to 40 ppt. It thus appears that *S. ocellatus* responds to abrupt salinity changes somewhat differently than does *C. variegatus*, as metabolism increased upon transfer to 50 ppt in *S. ocellatus*, but decreased in *C. variegatus*. This difference may be due to the smaller magnitude of the salinity changes performed by Wakeman and Wohlschlag (1983) (10 ppt vs 20 to 30 ppt in the present study). However, these results could also be attributable to different sizes and activity patterns of experimental fish between the two studies.

In a similar study, Shushmin (1989) studied the dynamics of oxygen consumption in juvenile golden mullet, *Liza aurata*, following abrupt transfers from 18 ppt to a series of salinities ranging from 0.4 ppt to 50 ppt. All salinity changes resulted in increased metabolic rates for one to three days following the transfer, with metabolism generally returning to near normal levels within three to four days. It is not surprising that salinity changes are more stressful in the golden mullet, since the species has lower salinity tolerance and osmoregulatory ability than *C. variegatus*.

Barton and Barton (1987) examined metabolism in juvenile (< 1 g) *C. variegatus* collected from inland saline lakes of San Salvador Island. They measured metabolic rates of

fish at 10 ppt and 35 ppt following abrupt transfer from an acclimation salinity of 35 ppt. Metabolic rates in fish measured at 10 ppt were significantly higher than those made at the acclimation salinity. These results are somewhat different from those obtained in the present study. However, measurements by Barton and Barton (1987) were made only 3 h following abrupt transfer to the reduced salinity, whereas measurements in the present study were made 6-12 h following a gradual change in salinity. Fish in this study thus experienced a less stressful transition to the new salinity. An ontogenetic effect may also account for the observed differences, as many investigations have demonstrated that adults and juveniles of the same species may differ greatly in physiological competence (e.g., Kinne, 1966; Martin, 1968; Oikawa et al., 1991).

Other relevant comparisons can be made with data on juvenile croaker, *Micropogonias undulatus*, and juvenile spot, *Leiostomus xanthurus* (Moser and Gerry, 1989; Moser and Miller, 1994). These studies examined effects on metabolism from fluctuations in salinity between 0 ppt and 35 ppt. Like *C. variegatus*, a salinity decrease from higher salinities to 0 ppt generally led to a decreased metabolism in *L. xanthurus*. Similarly, when fish were acclimated to 0 ppt and salinity was increased, metabolism was elevated at the higher salinities. Contrary to findings for *C. variegatus*, salinity fluctuations between 15 ppt and 35 ppt elicited changes in metabolism in both *L. xanthurus* and *M. undulatus*. Thus, these species are influenced to a greater extent by salinity fluctuations than *C. variegatus*. However, rates of salinity fluctuation in the above studies were faster than rates used in the present study (16 or 32 ppt h^{-1} vs 3.3 or 5 ppt h^{-1}), and *C. variegatus* likely experiences greater fluctuations in salinity than do spot or croaker.

Acclimation state is the most important measured factor influencing the metabolic response of *C. variegatus* to simulated tidal changes in salinity. However, direction of the salinity change also influenced metabolism in *C. variegatus*. Like the results obtained by Moser and Miller (1994) on juvenile *L. xanthurus*, it appears that *C. variegatus* adjusts to increasing salinity more effectively than to decreasing salinity, as evidenced by the

responses to salinity changes between 20 ppt and 2 ppt, and between 50 ppt and 30 ppt. Thus, when salinity is increased over the course of the trial, fish are only affected metabolically at the very highest and lowest salinities. However, when fish are acclimated to a high salinity, and salinity is then decreased to a more typical range, individuals take advantage by increasing metabolism to more normal levels.

Thus, *C. variegatus* is well adapted to a varying salinity environment. Its metabolism is unaffected by changes in salinity over the typical range encountered, even when salinity is changed rapidly. Furthermore, this corroborates my hypothesis that *C. variegatus* tolerates extremes in salinity by lowering metabolism and decreasing energy expenditures. Fish appear to wait for conditions to improve, and respond to these more favorable conditions by returning metabolism to normal levels.

A decrease in energetic expenditures as just described is a potentially adaptive response for fishes living in variable salinity environments like those of Florida coastal salt marshes. While few data exist on responses of other salt marsh residents to wide ranges in salinity, I suspect this may be a general pattern. *Cyprinodon variegatus* may be unusual because of its broad tolerances, but is a useful experimental animal because of this as well. Information gleaned from studies with *C. variegatus* may indicate areas of examination for other important salt marsh teleosts which may have more limited salinity tolerance, but which may follow the same general metabolic patterns seen in *C. variegatus*.

CHAPTER 4
INFLUENCE OF A FLUCTUATING SALINITY REGIME ON OSMOREGULATION IN *CYPRINODON VARIEGATUS*

Introduction

Few areas in the field of fish physiology have received as much attention as the study of osmoregulation. The basic patterns of osmoregulation are well understood and are reviewed extensively by Evans (1984), Karnaky (1986), Ventrella et al. (1992), Evans (1993), McCormick (1994), and Wood and Marshall (1994). The mechanisms of osmoregulation in euryhaline fish are not unusual. Euryhaline fishes are unique in having the ability to osmoregulate efficiently in waters of highly variable salinity. They can osmoregulate in water both more and less concentrated than their own body fluids, and they are able to alter their pattern of osmoregulation rapidly if they live in environments where sudden fluctuations in salinity may occur.

Despite the voluminous research done in this area, few studies have examined patterns of osmoregulation in fishes that may encounter salinities outside the range from freshwater (0 ppt) to seawater (35 ppt). Such species tend to be small and of little or no economic importance, with a notable exception being the milkfish, *Chanos chanos* (Ferraris et al., 1988; Swanson, 1991). However, to understand osmoregulation patterns in fishes, studies need to encompass the range of salinities encountered by species in nature.

One topic that has received little attention concerns the effect of fluctuating salinities on osmoregulation in teleosts. Salinity is a limiting factor in certain estuarine and salt marsh habitats, and may widely vary daily and seasonally. Despite this, most investigations of osmoregulation in fishes involve studies at constant salinities. While such studies provide

important information on overall osmoregulatory patterns, they provide less information about more ecologically relevant responses.

The aim of the present investigation was twofold. First, I examined the ability of individuals of the euryhaline teleost, *Cyprinodon variegatus*, to regulate plasma osmolality under the influence of a cycling salinity regime. Second I examined a hypothesis proposed by Goolish and Burton (1988) in a study involving the intertidal copepod *Tigriopus californicus*. Goolish and Burton (1988) suggested that species exposed to fluctuating salinities would be able to respond more rapidly and completely to salinity stress. In other words, could past exposure to changing salinity result not in improved osmoregulation at any single salinity, but rather to improved performance immediately following another salinity fluctuation? These hypotheses were examined by determining plasma osmolality and hematocrit of individual *C. variegatus* subjected to fluctuations in salinity over a wide range of ambient salinities.

Methods

Collections of fish used in this study were obtained from tidal creeks in the salt marsh near Cedar Key, Florida. Specimens were transported back to the laboratory in 128 L coolers supplied with aeration and filled with water obtained from the collection site. Fish were obtained in two collections made during September 1994. The salinity of the collection site was approximately 25 ppt for both collections. This study was conducted at the Southeastern Biological Science Center (SBSC), National Biological Service, Gainesville, Florida. Fish were held overnight in the coolers used for transportation (with aeration) before transfer to a 1.2 m diameter fiberglass holding tank containing water at 30 ppt. Fish were treated prophylactically for 7-14 days in a 5 mg L^{-1} solution of Acriflavine®. Following treatment, all fish were transferred to experimental aquaria (30 ppt salinity = 860 mOsm kg^{-1}) located within a constant environment room maintained at 20 ± 1 °C and on a

12:12 h light:dark cycle. Both holding and experimental aquaria were equipped with sponge filters providing continuous aeration, and fish were fed flake food once each day.

Experimental aquaria were used to subject fish to a cycling salinity regime. Seven salinity trials were performed (Table 4-1). All fish were maintained in experimental aquaria located on bank 1 for eight days prior to initiating the first salinity change. Salinity changes were effected by carefully netting fish from the experimental aquaria located on bank 1, and immediately transferring individuals into the appropriate experimental aquaria located on bank 2. Fish remained in experimental aquaria located on bank 2 for 24 h before transfer back to tanks located on bank 1. Fish then remained in bank 1 experimental aquaria for 24 h before again being transferred to aquaria on bank 2. Thus, one complete cycle began with 24 h in bank 2 aquaria, and ended following 24 h in bank 1 aquaria. The procedure was repeated so that a total of 10 cycles was performed (20 days). A subsample of fish was removed for testing from each experimental aquarium just prior to the first change in salinity (cycle 0), and at the end of cycles 1 (day 2), 5 (day 10), and 10 (day 20). All subsamples were thus taken following a 24 h period in bank 1 aquaria (30 ppt).

Following completion of the 10th cycle, fish remaining in all decreasing salinity groups (groups D_1, D_2, and D_3) were transferred to aquaria at 2 ppt and fish remaining in all increasing salinity groups (groups I_1, I_2, and I_3) were transferred to aquaria at 60 ppt. Fish in the aquaria maintained at 30 ppt were split into three groups at that time: one third of the group was transferred to 2 ppt (group C_D), one third transferred to 30 ppt (group C), and the final third transferred to 60 ppt (group C_I). Fish remained in these salinities for 24 h and were then removed for testing (day 21). Salinities were checked daily with a Leica® temperature compensated refractometer and adjusted as necessary.

Hematocrit (Hct) and plasma osmolality were determined at each sampling interval. Fish were first carefully netted from their experimental aquaria and blotted dry. Blood was taken by sternal cardiac puncture using heparinized microhematocrit tubes drawn to a fine point, and fish were weighed and standard length determined. The tubes were then

Table 4-1. Salinity trials used in cyclical salinity study. The group maintained at 30 ppt was split into three groups following cycle 10 (day 20); groups C_D, C, and C_I (see text for details).

Group	Direction of Salinity Change	Salinity in Bank 1 (ppt)	Salinity in Bank 2 (ppt)
D_1	Decreasing Salinity (n=25)	30	2
D_2	Decreasing Salinity (n=25)	30	10
D_3	Decreasing Salinity (n=25)	30	20
C, C_D, and C_I	No Change (n=35)	30	30
I_1	Increasing Salinity (n=25)	30	40
I_2	Increasing Salinity (n=25)	30	50
I_3	Increasing Salinity (n=25)	30	60

centrifuged in a micro-hematocrit centrifuge for 10 minutes to separate plasma from cells. Hematocrit was read using a micro-capillary reader before plasma was isolated from formed elements by scoring the tube with a file and retaining only the portion containing plasma. Plasma osmolality (mOsm kg^{-1}) was determined on 5 µl samples using a Wescor® 5500 vapor pressure osmometer. Fish were used without regard to sex, and all blood samples were taken between 0600 h and 1000 h. Statistical procedures followed Winer et al. (1991) and Sokal and Rohlf (1995). All statistical analyses were one way tests using the Tukey-Kramer post hoc comparison ($p = 0.05$)

Results

All fish entered into the experimental procedure survived the entire duration of the experiment. Fish in all groups ate normally throughout the course of the experiment, and no discernible changes in behavior were observed during the experimental procedure for any

group. Fish appeared to have no difficulty in tolerating the imposed salinity fluctuations, even in groups D_1 and I_3, which were subjected to daily changes in salinity of 28 ppt and 30 ppt, respectively. The influence of body mass on both Hct and plasma osmolality was evaluated using an analysis of covariance, and for no analysis was body mass a significant covariate. Sizes of fish used in this study ranged from 0.75 g to 5.887 g (2.8 mm to 5.8 mm standard length, mean 4.1 ± 0.038 mm), with a mean mass of 2.245 ± 0.066 g. Subsamples of groups were not significantly different in mass.

Hematocrit and plasma osmolality values were compared both between salinity trials for each time period sampled (i.e., all groups were compared to one another on days 0, 2, 10, 20, and 21), and within a single trial over the time course of the experiment. Groups experiencing increases in salinity (groups I_1, I_2, I_3, and C_I) were compared separately from groups experiencing decreases in salinity (groups D_1, D_2, D_3, and C_D), with all compared to the group maintained at a constant salinity (group C). Data from days 0 through 20 are discussed separately from day 21 data as follows.

Day 0 through 20

Hematocrit values showed no obvious trends either between salinity trials or within any single trial over the time course of the experiment (Table 4-2). There were no statistically significant differences ($p > 0.05$) within any group (between days 0 and 20), or among groups on days 0, 2, 10, or 20. There was a slight trend towards increased variance in Hct values over the course of the experiment in all groups, peaking at cycle 5, but differences were non-significant in all cases.

Plasma osmolality data, unlike the Hct data, exhibited consistent trends. No significant differences existed among groups on days 0, 2, 10, or 20. However, comparisons within groups over time revealed a different pattern. All groups, regardless of salinity regime, showed a trend towards slightly elevated plasma osmolality between days 0 and 20 (Table 4-2; Figures 4-1 and 4-2). For groups C and I_1, this was statistically significant, with

Table 4-2. Results of salinity fluctuations experiment. Values in the top row of each cell represent hematocrit measurements (% erythrocytes), values in bottom row of each cell represent plasma osmolality measurements (mOsm kg^{-1}). Sample sizes are n=5 for each cell. All values are expressed as means ± se. See text for explanation of group abbreviations.

DAY	GROUP								
	D_1	D_2	D_3	C_D	C	C_I	I_1	I_2	I_3
0	24.6 ± 0.68	26.5 ± 0.96	26.4 ± 1.12	-----	25.2 ± 0.92	-----	25.3 ± 1.32	23.8 ± 0.49	25.0 ± 0.63
	358.5 ± 3.32	364.3 ± 7.78	367.3 ± 2.77	-----	364.8 ± 2.33	-----	359.75 ± 8.6	372.7 ± 3.75	375.6 ± 12.7
2	22.8 ± 1.39	24.8 ± 0.97	23.6 ± 1.03	-----	22.0 ± 0.95	-----	26.0 ± 1.38	27.0 ± 1.64	27.0 ± 1.23
	363.3 ± 6.92	379.2 ± 7.97	370.7 ± 10.9	-----	364.5 ± 6.60	-----	380.7 ± 11.2	378.1 ± 7.85	382.4 ± 6.60
10	26.8 ± 1.50	22.8 ± 1.93	23.8 ± 1.28	-----	26.8 ± 1.39	-----	23.0 ± 2.12	28.2 ± 3.25	22.8 ± 2.87
	386.1 ± 7.36	382.6 ± 7.72	379.5 ± 12.0	-----	375.2 ± 3.16	-----	384.6 ± 10.2	389.7 ± 5.48	397.7 ± 9.48
20	24.8 ± 1.02	23.8 ± 1.66	26.0 ± 2.59	-----	24.6 ± 1.40	-----	27.8 ± 2.63	26.4 ± 1.69	24.6 ± 1.69
	395.5 ± 7.21	371.6 ± 8.21	403.9 ± 8.20	-----	390.2 ± 2.78	-----	389.8 ± 8.48	398.5 ± 6.69	380.0 ± 7.75
21	26.2 ± 0.49	25.0 ± 1.82	28.8 ± 1.83	25.0 ± 0.84	24.4 ± 1.97	29.2 ± 0.37	27.4 ± 1.12	24.6 ± 1.21	24.2 ± 0.86
	367.2 ± 6.81	362.3 ± 9.19	373.1 ± 7.95	336.4 ± 6.48	395.2 ± 4.79	556.6 ± 5.88	436.3 ± 9.3	413.8 ± 9.29	420.8 ± 7.51

Figure 4-1. Mean plasma osmolality values measured for groups experiencing decreases in salinity during the course of the experiment. Group designations are as follows: D_1, salinity fluctuated between 30 ppt and 2 ppt; D_2; salinity fluctuated between 30 ppt and 10 ppt; D_3, salinity fluctuated between 30 ppt and 20 ppt; C_D; salinity constant at 30 ppt for days 0-20, decreased to 2 ppt following subsample on day 20; C, salinity constant at 30 ppt. Sample sizes are n=5 for each group. See text for details of experimental procedure.

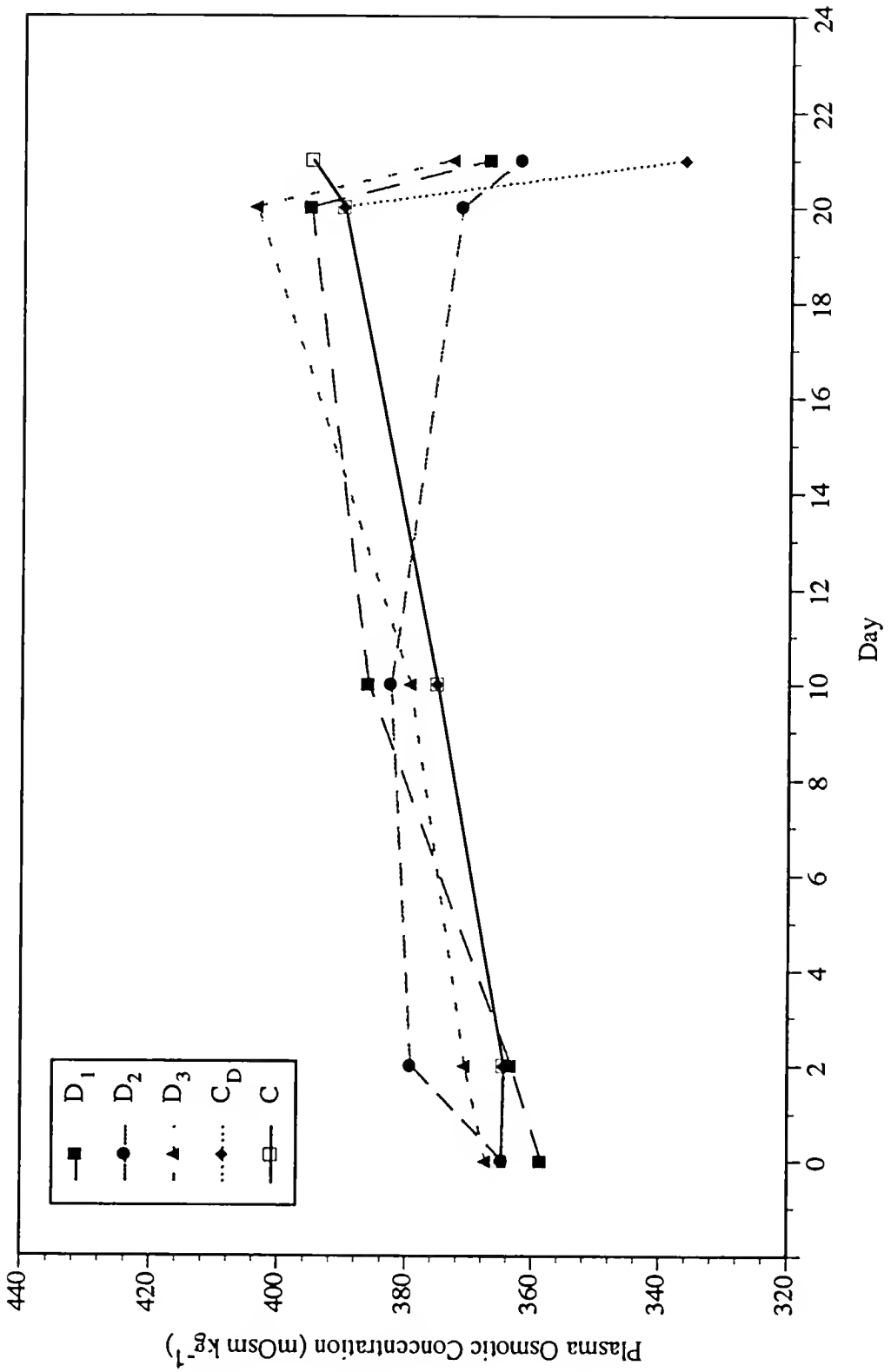
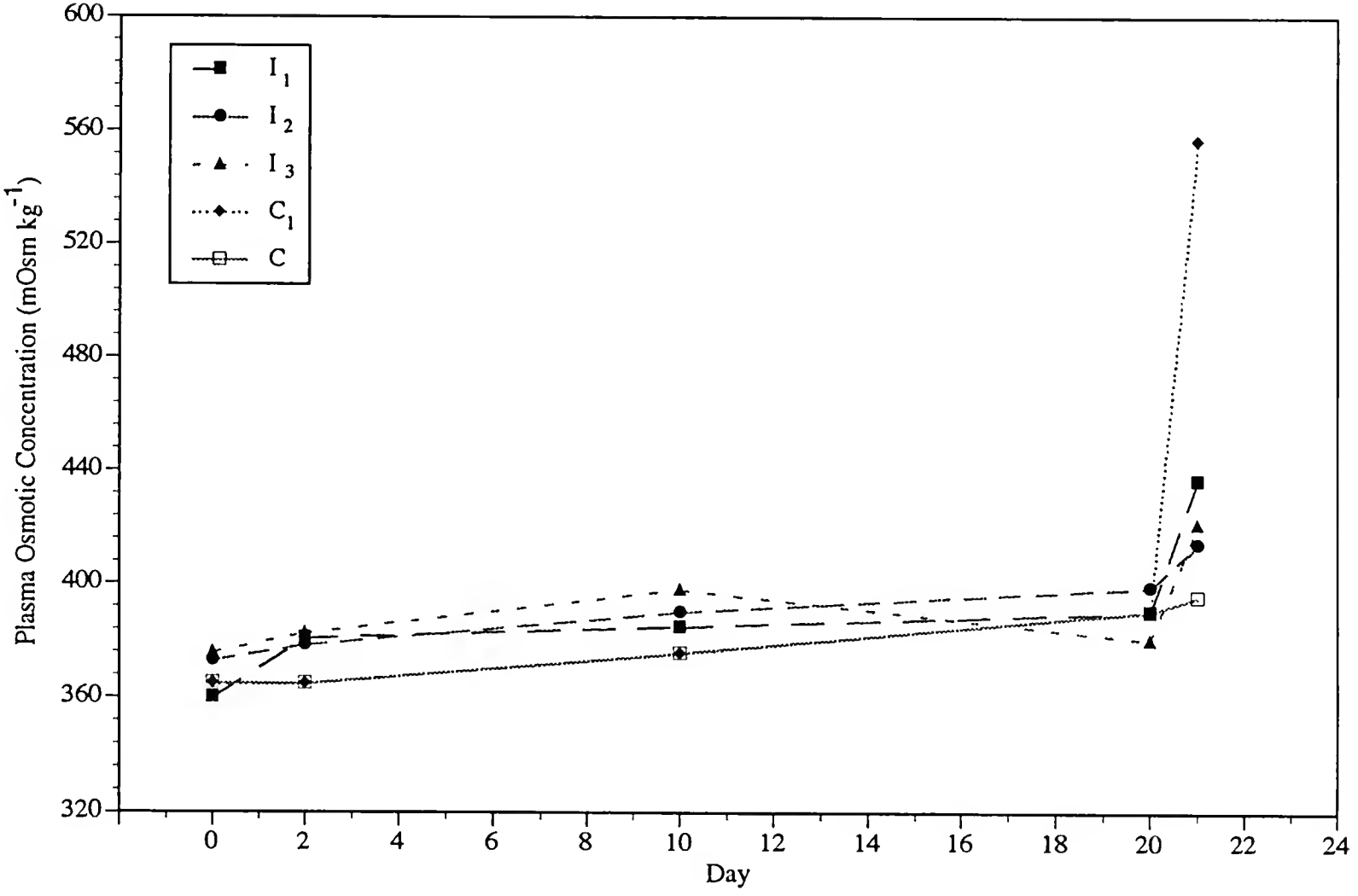

Plasma Osmotic Concentration (mOsm kg-1)
440
420
400
380
360
340
320
0
2
4
6
8
10
12
14
16
18
20
22
24
Day
D1
D2
D3
CD
C

Figure 4-2. Mean plasma osmolality values measured for groups experiencing increases in salinity during the course of the experiment. Group designations are as follows: I_1, salinity fluctuated between 30 ppt and 40 ppt; I_2; salinity fluctuated between 30 ppt and 50 ppt; I_3, salinity fluctuated between 30 ppt and 60 ppt; C_1; salinity constant at 30 ppt for days 0-20, increased to 60 ppt following subsample on day 20; C, salinity constant at 30 ppt. Sample sizes are n=5 for each group. See text for details of experimental procedure.


600
560
520
480
440
400
360
320
Plasma Osmotic Concentration (mOsm kg-1)
I1
I2
I3
C1
C
0
2
4
6
8
10
12
14
16
18
20
22
24
Day

the values on day 20 significantly higher than values from day 0 and day 1 ($p<0.05$); all other groups exhibited the same trend, but differences were statistically non-significant. This appears to be related to the experimental manipulation of fish, and not to the salinity regime experienced by each group. All groups regulated plasma osmolality effectively. No differences in regulatory ability could be seen among any of the groups tested, regardless of the magnitude or direction of the salinity fluctuation.

Day 21

The above addresses the question of how effectively *C. variegatus* regulates plasma osmolality in the face of salinity fluctuations. Transfer of individuals to either 2 or 60 ppt following 20 days of exposure to a variety of salinity fluctuations addresses the second question posed earlier: Does past exposure to large salinity fluctuations result in improved osmoregulatory performance, compared to animals experiencing little or no salinity fluctuation, immediately following a fluctuation in salinity? All measurements here were taken 24 h (on day 21) following the final salinity fluctuation.

Hematocrit results showed no consistent trends on day 21 samples. Only one significant difference was noted, with the Hct value for group C_1 being significantly elevated compared to the value for group C ($p<0.05$). All other comparisons showed no significant differences in Hct.

There were significant differences in plasma osmolality for day 21 measurements. Here groups which had experienced greater fluctuations in salinity over the course of the experiment showed a much better ability to osmoregulate compared to the control group or groups which had undergone small salinity fluctuations. Increasing salinity seemed to have a greater impact on plasma osmolality than did decreasing salinity (Figures 4-1 and 4-2).

Plasma osmolality in groups D_1, D_2, and D_3 all exhibited declines on day 21 relative to day 20, but these differences were not statistically significant. No differences in regulatory ability among these three groups, or with the control group (group C), could be

discerned. However, group C_D, which had been maintained at 30 ppt for the first 20 days of the experiment, did show a significant decrease in plasma osmolality on day 21 relative to values measured on days 0 through 20. Furthermore, this group had lower osmolality values on day 21 than all other groups, although this difference was only statistically significant when compared to groups C and D_3.

More striking differences existed for groups I_1, I_2, I_3, and C_I. In all cases plasma osmolality was elevated on day 21 relative to measurements taken from the same group on day 20 or earlier. Group I_3, which had experienced daily fluctuations in salinity of 30 ppt, showed the smallest increase in osmolality values, being significantly higher than measurements from day 0 only. Group I_2 showed a similar result, with the plasma osmolality value from day 21 significantly higher than values from day 0 and day 2 only. Groups I_1 and C_I showed the largest changes in plasma osmolality on day 21. Both groups had osmolality values that were significantly higher than all previous measurements taken from their respective groups. The value for group I_1 was elevated compared to groups I_2, I_3, and C, although this increase was statistically significantly only when compared to group C. Group C_I was significantly elevated when compared to all other groups (including group I_1).

Discussion

The ability to adjust rapidly to altered salinities would be an obvious advantage to salt marsh organisms. Physiological responses of euryhaline fishes exposed to rapid changes in salinity can be grouped into two phases (Holmes and Donaldson, 1969): an adaptive period and a regulatory period. During the adaptive period, plasma osmolality varies, gradually returning to values approaching original levels. In the regulatory period, plasma osmolality is more finely controlled as the fish adjusts to the altered salinity and reaches ionic homeostasis. Fishes which reach the regulatory period quickly (i.e., have short adaptive periods) should be best able to tolerate alterations in ambient salinity. Although the

length of the adaptive period was not measured in the present study, results nevertheless suggest that it is relatively short for *C. variegatus*.

Nordlie (1985) showed that *C. variegatus* was an excellent osmotic regulator over a salinity range from 0.3 ppt to 70 ppt. Plasma osmolality values changed only slightly over this range of salinities in his study, varying by only 40 mOsm kg^{-1}. However, fish in his study were fully acclimated to each experimental salinity prior to testing. The present study indicates that *C. variegatus* is an excellent regulator of plasma osmolality even when fishes are exposed to large daily fluctuations in salinity. Although fish in all groups in the present study regulated at slightly higher levels than seen by Nordlie (1985), plasma osmolality values varied similarly, with differences of less than 40 mOsm kg^{-1} for all groups on days 0 through 20. The transfer process itself elicited much of this variation, with slight increases in plasma osmolality seen over the time course of the experiment. A similar trend was seen in a study by Woo and Wu (1982) on the red grouper, *Epinephelus akaara*, and the black sea bream, *Mylio macrocephalus*.

The influence of a single alteration in salinity on osmoregulatory ability has been studied for a number of fishes, with changes in plasma osmolality observed in the present study of similar magnitude to those seen in other euryhaline fishes (Wakeman and Wohlschlag, 1983; Engel et al., 1987; Ferraris et al., 1988; Mancera et al., 1993; Yoshikawa et al., 1993; Altimiras et al., 1994; Tort et al., 1994). Few studies have examined how fluctuating salinities influence osmoregulation of fishes. The best comparison of my results are with a study on *Blennius pholis* by Davenport and Vahl (1979). In their study *B. pholis* were exposed to alternating periods of freshwater and seawater, with each period lasting 6 h. Similar to the results from the present study, plasma osmolality did not vary significantly over the course of the experiment.

No consistent differences in Hct were observed over the course of the experiment. Nordlie et al., (1995) found that *C. variegatus* efficiently regulates water content over a wide range of salinities, with a difference of only 4% observed between 0 ppt and 100 ppt.

These results suggest that there are no large movements of water between the blood and tissues as a result of fluctuations in salinity over the range tested. The lack of response in hematocrit in this study may also indicate that within the range of salinities studied *C. variegatus* is subject to normal, or tolerance physiological processes, as an increase in hematocrit values would be expected if *C. variegatus* were exposed to conditions leading to resistance processes (Swift, 1982).

It has been hypothesized that fishes occurring in a habitat where salinity often fluctuates may be able to respond more quickly and completely to alterations in salinity, and that the limits of tolerance are farther apart if salinity fluctuates periodically (Gunter, 1967; Spaargaren, 1974; Johnston and Cheverie, 1985). My results provide evidence that prior exposure to fluctuations in salinity does impart an osmoregulatory advantage. Fishes previously exposed to large fluctuations in salinity regulated plasma osmolality better than fishes that had previously experienced no change or small changes in salinity. Increasing salinity had the greatest impact on regulation of plasma osmolality. Group C_I, which had experienced no prior change in salinity, and group I_1, exposed to the smallest fluctuations in salinity prior to being transferred to 60 ppt showed large increases in plasma osmolality compared to the control group and groups which had previously experienced large fluctuations in salinity. Decreasing salinity groups exhibited the same pattern, but differences in osmolality were less pronounced. Only group C_D, which had not been previously exposed to fluctuations in salinity, showed a significant decline in plasma osmolality after transfer to 2 ppt. Salinities between 2 ppt and 35-40 ppt are typically encountered by this population of *C. variegatus* in its native habitat, with salinities as high as 60 ppt rarely encountered. Thus, it is not surprising that transfer to 60 ppt elicited greater changes in regulation of plasma osmolality.

Variations in salinity imposed on fish in this study caused no dramatic effects. No adverse behavior or mortalities were noted throughout the course of the experiment. Thus, despite the differences seen in regulation of plasma osmolality between some of the

experimental groups, these differences were not large enough in magnitude to cause observable distress in the experimental animals. These results indicate that *C. variegatus* is well adjusted for life in variable salinity conditions. It would be interesting to compare results from the present experiments with studies examining the influence of fluctuating salinity on osmoregulation in fishes that can tolerate changing salinity, but that experience infrequent salinity variations in their native habitat.

CHAPTER 5
INFLUENCE OF ENVIRONMENTAL SALINITY ON BLOOD OXYGEN LEVELS OF *CYPRINODON VARIEGATUS*

Introduction

The sheepshead minnow, *Cyprinodon variegatus*, is a euryhaline teleost whose typical habitats are brackish water, coastal salt marshes that experience frequent salinity fluctuations. Variations in environmental salinity may directly affect the respiratory system of fishes in at least two ways: by affecting the solubility of oxygen in the water pumped over the gills, and by affecting the solubility of oxygen dissolved in plasma. Changes in the ionic composition of bodily fluids may also interact with oxygen to influence tolerance to variable salinity conditions (Truchot, 1987). Furthermore, fishes in saline water with low oxygen tension must balance maximizing branchial oxygen diffusion with greater osmoregulatory demands due to the accompanying increases in ion and water exchange (Perry and McDonald, 1993). Additionally, the oxygen content of many aquatic habitats is subject to large natural fluctuations, so oxygen is a potentially limiting factor by itself (Dejours, 1987; Graham, 1990). This is especially true in shallow waters, where chronic or periodic hypoxia may be a common phenomenon (Graham, 1990). Salt marsh habitats are often exposed to hypoxic conditions (Renaud, 1985; Toulmond, 1987).

Cyprinodon variegatus is an extremely competent euryhaline teleost. Over a range of salinities from freshwater (0 ppt) to 40 ppt, very small changes in metabolism occur (Nordlie, et al., 1991; this study, Chapter 2). Both oxygen consumption and critical oxygen tension (P_c) are essentially unaffected by changes in salinity over this range. Salinities above this range cause metabolic adjustments, with increases in P_c and decreases in metabolism observed (this study, Chapter 2). The mechanisms involved in maintaining

constant metabolism and P_c at low to moderate salinities, and the alterations of metabolism and P_c at higher salinities likely involve adjustments in oxygen transport. Not only do high salinity waters have lowered concentrations of dissolved oxygen, but salinity itself seems to elicit physiological responses similar to those resulting from lowered levels of oxygen. Increasing blood oxygen levels with increasing salinity, possibly leveling off at the highest salinities, could lead to the observed responses in metabolism and P_c in *C. variegatus*.

Increased blood gas transport is likely the primary mechansim used by most fishes to increase the amount of oxygen delivered to tissues. Blood oxygen transport in most teleosts is dependent upon the respiratory pigment hemoglobin. Blood oxygen transport is normally increased by increasing the concentration of hemoglobin, increasing the number of erythrocytes in circulation, and/or adjusting the affinity of hemoglobin for oxygen (Davis, 1975; Wells et al., 1989; Jensen et al., 1993; Perry and McDonald, 1993). I examined hemoglobin concentration, erythrocyte count, and packed cell volume (hematocrit) over a wide range of salinities for individuals of *C. variegatus* to evaluate the influence of salinity on blood oxygen levels in this extremely euryhaline species.

Methods

Collections of fish, transportation back to the laboratory, and general lab procedures were performed as described previously. Using the same protocol described earlier, fish were sequentially acclimated to a series of salinities ranging from 0 ppt to 80 ppt (0 to 2285 mOsm kg^{-1}). At the end of the acclimation period, fish were sacrificed to determine hemoglobin concentration ([Hb]), hematocrit (Hct), and erythrocyte (RBC) count.

Blood Sampling

Fish were first carefully netted from their experimental aquaria and blotted dry. Blood was taken by sternal cardiac puncture using freshly heparinized microhematocrit tubes drawn to a fine point. Blood from each fish was collected in two microhematocrit

tubes. Once the first tube was filled with a volume > 20 µl, the blood was immediately dispensed into a small ceramic crucible. Eppendorf® micropipettes were then used to dispense aliquots of blood from the crucible into test tubes used in the determination of hemoglobin concentration and erythrocyte count. A second microhematocrit tube was then filled and used for determination of the hematocrit, and mass and standard length of fish were then determined. Fish were used without regard to sex, and all blood samples were taken between 0700 h and 1100 h. Food was withheld from experimental aquaria for 24 h prior to testing to ensure that all fish were post-absorptive. Statistical procedures followed Winer et al. (1991) and Sokal and Rohlf (1995). All statistical analyses were one way tests using the Tukey-Kramer post hoc comparison ($p = 0.05$).

Hemoglobin Analysis

Hemoglobin concentration was measured spectrophotometrically on 10 µl samples using the cyanomethemoglobin method (Brown, 1993). As recommended by Innes and Wells (1985), the cyanomethemoglobin solutions were centrifuged for 10 min at 5000 *g* prior to colorimetric determination to remove erythrocyte debris. This method gives reliable results when used with fish blood (Blaxhall, 1972; Blaxhall and Daisley, 1973; Coburn, 1973; Innes and Wells, 1985).

Erythrocyte Count

Erythrocytes were counted immediately following dilution in test tubes containing Natt and Herricks solution (Campbell and Murru, 1990). This solution acts as both stain and dilutent and is routinely used for counting erythrocytes of fish (Campbell and Murru, 1990). A 1:200 dilution was used and cells were counted in an improved Neubauer hematocytometer following precautions outlined in Brown (1993).

Hematocrit

Hematocrit was measured to determine the packed cell volume of the erythrocytes contained in the blood. Immediately after filling the second microhematocrit tube one end was sealed and the tube placed into a micro-hematocrit centrifuge. Tubes were then centrifuged for 10 min to separate plasma from formed elements. The hematocrit was read using a micro-capillary reader and expressed as percent erythrocyte.

From the test values obtained, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated for each fish as follows (Brown, 1993):

$$MCV = \frac{Hct \times 10^3}{RBC/l}, \quad MCH = \frac{[Hb]\ (g/l)}{RBC/l}, \quad MCHC = \frac{[Hb]\ (g/dl)}{Hct}$$

These erythrocyte indices are used to further define the relationship between hemoglobin content and size of the erythrocyte.

Results

The various measures of blood oxygen are arranged by salinity group in Table 5-1. Significant differences over the range of test salinities were found for all parameters except MCHC. Body mass had no significant influence on any of the measured or calculated blood oxygen indices.

Salinity exerted the greatest influence on erythrocyte count (Figure 5-1). Values obtained at 80 ppt and 0 ppt were significantly higher than all other salinities. Erythrocyte count was next highest at 60 ppt and 70 ppt, being significantly elevated compared to values in fish acclimated to 2, 10, 20, and 30 ppt. Fish acclimated to salinities from 2 ppt through 50 ppt exhibited no significant differences in erythrocyte count.

Measurements of hemoglobin concentration exhibited a similar pattern, although fewer significant differences were noted (Figure 5-2). Mean values of fishes acclimated to 0 ppt were highest and significantly different from fish acclimated to salinities from 2 ppt

Table 5-1. Hematocrit (Hct), hemoglobin concentration ([Hb]), erythrocyte count (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) as a function of salinity for *Cyprinodon variegatus*. All values are expressed as means ± se. See text for further details on blood indices.

Salinity (ppt)	*n*	Hct (%)	[Hb] (g dl^{-1})	RBC (x 10^6 mm^{-3})	MCH (pg)	MCV (μm^3)	MCHC (%)
0	9	29.56 ± 0.90	7.52 ± 0.35	2.79 ± 0.06	26.93 ± 1.08	106.23 ± 3.78	25.52 ± 1.10
2	12	20.33 ± 1.28	5.44 ± 0.22	1.91 ± 0.07	28.50 ± 0.83	105.76 ± 4.90	27.51 ± 1.49
10	10	23.80 ± 1.55	5.23 ± 0.29	1.88 ± 0.12	28.08 ± 1.37	128.51 ± 8.51	22.34 ± 1.13
20	9	19.56 ± 1.19	4.73 ± 0.23	1.71 ± 0.05	27.68 ± 1.15	113.62 ± 4.65	24.69 ± 1.38
30	10	21.10 ± 0.89	5.55 ± 0.39	1.88 ± 0.14	29.80 ± 1.64	114.92 ± 5.04	26.13 ± 1.22
40	12	22.83 ± 1.22	5.85 ± 0.20	2.16 ± 0.06	27.13 ± 0.52	106.58 ± 6.13	26.20 ± 1.35
50	10	22.5 ± 1.21	5.82 ± 0.35	2.06 ± 0.07	28.39 ± 1.54	109.26 ± 4.26	26.39 ± 1.87
60	10	25.2 ± 1.67	6.75 ± 0.40	2.36 ± 0.08	28.45 ± 1.08	106.34 ± 5.05	27.26 ± 1.73
70	10	25.4 ± 1.87	6.09 ± 0.16	2.28 ± 0.08	26.89 ± 0.57	112.09 ± 8.30	24.88 ± 1.46
80	10	22.9 ± 1.27	6.43 ± 0.29	2.81 ± 0.08	23.04 ± 1.06	81.88 ± 4.40	28.89 ± 2.12

Figure 5-1. Mean erythrocyte (RBC) count over a range of salinities in *Cyprinodon variegatus* (bars indicate ± se; numerical values above the points in the figure indicate sample size at each salinity).

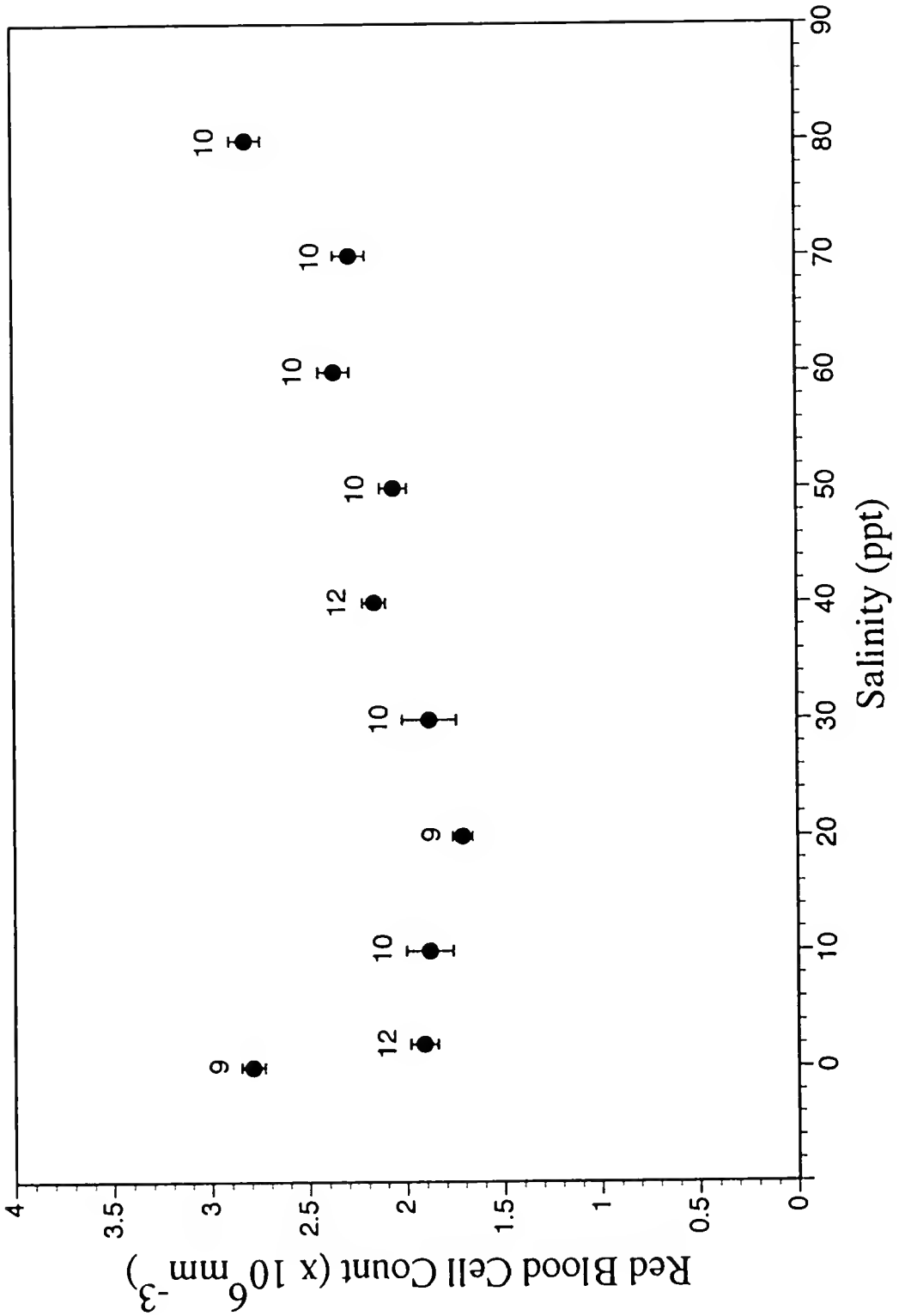
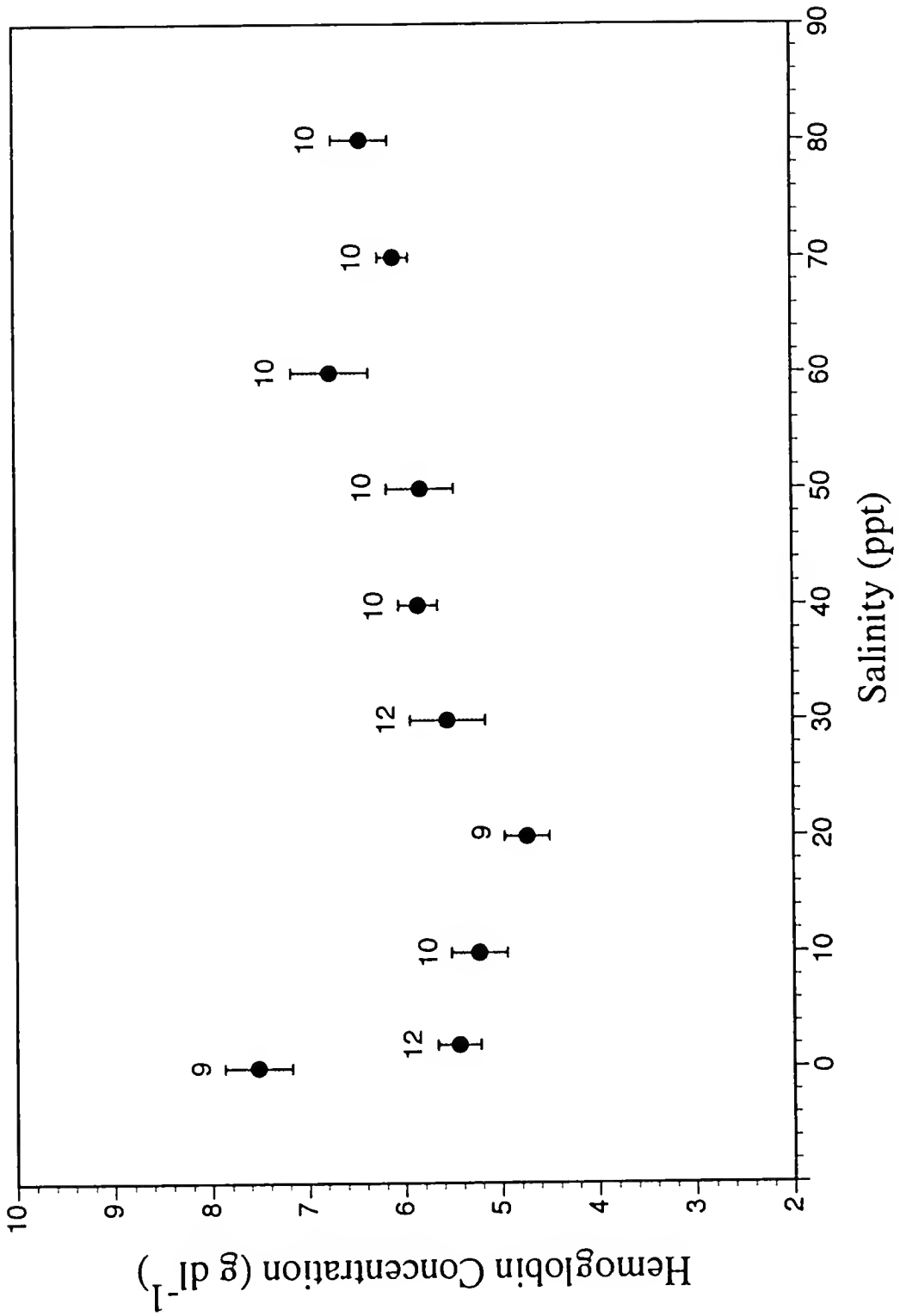

Red Blood Cell Count (x 10^6 mm^{-3})
Salinity (ppt)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
10
20
30
40
50
60
70
80
90
9
12
10
10
9
10
12
10
10
10
10

Figure 5-2. Mean hemoglobin concentration ([Hb]) over a range of salinities in *Cyprinodon variegatus* (bars indicate ± se; numerical values above the points in the figure indicate sample size at each salinity).


Hemoglobin Concentration (g dl-1)
Salinity (ppt)
10
9
8
7
6
5
4
3
2
0
10
20
30
40
50
60
70
80
90
9
12
10
9
12
10
10
10
10
10

through 50 ppt. Hemoglobin concentration was also elevated in fish acclimated to 60 ppt, 70 ppt, and 80 ppt, although these values were only significantly higher than the value of fish acclimated to 20 ppt.

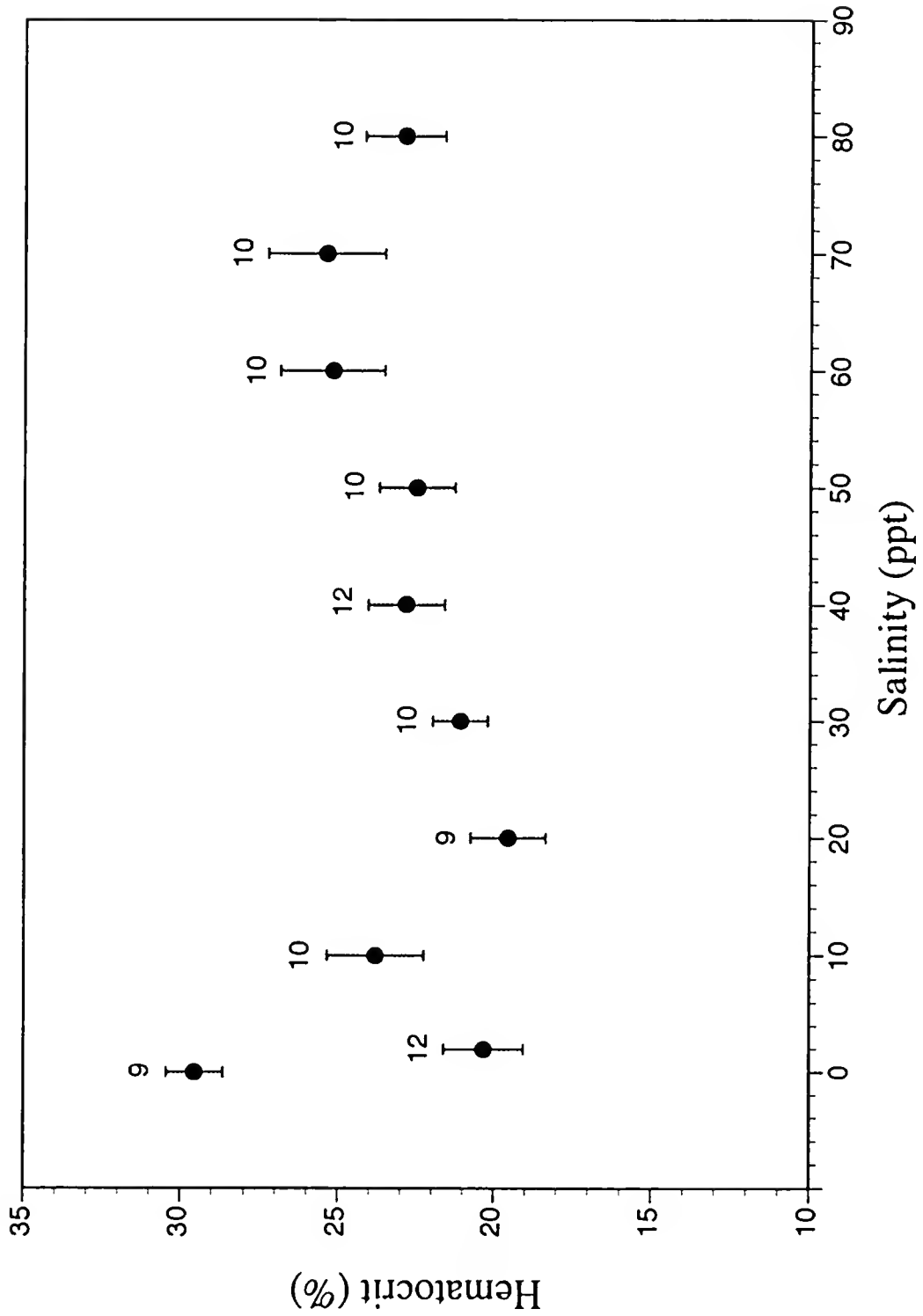
Hematocrit measurements showed less dependence upon salinity (Figure 5-3). Mean hematocrit was highest in 0 ppt, and was significantly different than mean values of fish acclimated to all salinities except 60 ppt and 70 ppt. No other significant differences in hematocrit were noted.

Calculated erythrocyte indices indicated a slightly different pattern. The average concentration of hemoglobin in the erythrocyte (MCHC) did not vary significantly among salinity acclimation groups. However, both the average weight of hemoglobin in the erythrocyte (MCH) and the average volume of the erythrocyte (MCV) were lowest in fishes acclimated to 80 ppt. MCH was significantly depressed in fish acclimated to 80 ppt when compared to groups acclimated to 2, 30, 60, and 70 ppt, with MCV values at 80 ppt significantly lower than values obtained for groups at 10, 20, 30, 50, and 70 ppt.

Discussion

Changes in environmental salinity can exert profound effects on blood oxygen transport. Increases in salinity confront fishes with the necessity of satisfying oxygen requirements under conditions of reduced oxygen availability. Fishes may exploit multiple strategies to optimize blood oxygen transport. The amount of oxygen delivered to the tissues by the blood per unit time is a product of the cardiac output, the oxygen tension difference between arterial and venous blood, and the blood oxygen capacitance coefficient (Jensen, 1991; Jensen et al., 1993). The capacitance coefficient reflects the hemoglobin's oxygen transporting properties, and can be adjusted in what have been termed 'qualitative' and 'quantitative' ways (Jensen, 1991). Regulation of hemoglobin-oxygen ($Hb\text{-}O_2$) affinity represents the primary method for qualitatively altering oxygen carrying capacity, with control of hemoglobin concentration the primary quantitative mechanism (Jensen, 1991).

Figure 5-3. Mean hematocrit (Hct) over a range of salinities in *Cyprinodon variegatus* (bars indicate ± se; numerical values above the points in the figure indicate sample size at each salinity).


Hematocrit (%)
35
30
25
20
15
10
0
10
20
30
40
50
60
70
80
90
Salinity (ppt)
9
12
10
10
9
10
12
10
10
10
10

Blood oxygen carrying capacity can also be increased quantitatively by release of stored erythrocytes, by accelerating maturation of immature erythrocytes, and/or by production of new erythrocytes (Murad et al., 1990); release of erythrocytes from storage organs (e.g., spleen) appears to be the most likely scenario (Soivio et al., 1980; Wells et al., 1989). Fish exposed to water of changing salinity would be expected to experience variability in their blood oxygen capacitance coefficient and blood oxygen carrying capacity (Jensen et al., 1993). Quantitative mechanisms for adjusting blood oxygen carrying capacity were examined in this study.

Few studies have examined the influence of salinity on oxygen carrying capacity of fishes. Guernsey and Poluhowich (1975) examined the blood oxygen capacity of American eels (*Anguilla rostrata*) acclimated to 0 ppt, 24 ppt, and 34 ppt. As in *C. variegatus*, hematocrit was highest in eels acclimated to 0 ppt. However, while oxygen capacity of acclimated eels was higher in 0 ppt than 34 ppt, the highest oxygen capacity was seen in eels acclimated to 24 ppt. In a similar study with the cichlid *Oreochromis niloticus*, Sun et al., (1995), observed a similar effect of salinity on measures of blood oxygen, with hemoglobin concentration significantly higher in 0 ppt than in higher salinities (5 to 20 ppt).

Other factors may also contribute to variations in oxygen carrying capacity of fishes. Hall and Gray (1929) were among the first to note that there is a general correlation between the habits of fishes and the hemoglobin concentration of their blood. More recent studies have shown that this generalization also applies to erythrocyte count and hematocrit (e.g., Haws and Goodnight, 1962; Coburn, 1973; Larsson et al., 1976; Putnam and Freel, 1978). In general, highly active fishes and those that regularly encounter hypoxic conditions have elevated blood oxygen carrying capacity relative to other species. Values determined for *C. variegatus* in the present study are comparable to other fishes with similar activity levels, and indicate that *C. variegatus* does not possess exceptionally high oxygen carrying

capacity at any salinity tested (Hattingh, 1972; Coburn, 1973; Larsson et al., 1976; Putnam and Freel, 1978; Smit and Hattingh, 1979; Pelster et al., 1988b).

Salinity had a significant effect on blood oxygen carrying capacity in *C. variegatus*. However, differences were seen only at the very highest and lowest salinities tested. As expected, oxygen carrying capacity increased at high salinities. Elevations were seen at 60 ppt through 80 ppt when compared to measures made at salinities between 2 ppt and 50 ppt. One finding of interest was indicated by MCV measurements. These results showed that at 80 ppt overall size of erythrocytes had decreased, implying the production and/or release of smaller, possibly immature erythrocytes. One possible explanation for this is that smaller erythrocytes have been found to be more effective in oxygen exchange than larger erythrocytes (Coburn, 1973) due to a larger surface area per unit volume, which may allow a faster rate of gas exchange. Energetic constraints related to viscosity of the blood may also play a role. A balance must exist between the advantages for oxygen transport from increased hematocrit with a disadvantage due to increased viscosity (Wells and Weber, 1991). Hematocrit began to increase as salinity reached 60 ppt, but declined back towards normal values at 80 ppt. It is possible that at extremely high salinities even small increases in hematocrit lead to significant viscosity problems. Utilizing smaller erythrocytes at extreme hypersalinities may help offset this problem.

Unexpectedly, oxygen carrying capacity was highest in the group acclimated to 0 ppt, as indicated by measurements of all blood indices. Although freshwater is tolerated by *C. variegatus*, previous work has shown that acclimation to freshwater is difficult and may result in significant mortality unless decreases in salinity are made in small steps (Nordlie and Walsh, 1989; Nordlie et al., 1991). Survival in freshwater requires many of the same responses that are necessary at extremely high salinities, with both freshwater and hypersaline conditions imposing difficult osmoregulatory problems for *C. variegatus*. In both situations, proliferation of mitochondria rich cells on gill epithelia is needed to maintain ionic balance (Evans, 1984; Evans, 1993; Wood and Marshall, 1994). However,

this extends the surface involved in ionic exchange at the expense of gill epithelia involved in gas exchange. Recent studies have shown that such proliferation of mitochondria rich cells does impair respiratory gas transfer (Bindon et al., 1994a; Bindon et al., 1994b). Mechanisms to increase oxygen carrying capacity of the blood would be expected under such conditions.

However, freshwater conditions differ significantly from hypersaline conditions in several ways. Most importantly, metabolism is reduced at extreme hypersalinities (this study, Chapter 2). In conjunction with elevated P_c, depressed metabolism at these hypersalinities greatly reduces energetic expenditures, partially alleviating the need for increased oxygen carrying capacity. Thus, whereas measures of blood oxygen are elevated at salinities of 60 ppt and higher, increases were moderated by a reduction in overall energetic expenditures. Fish acclimated to 0 ppt exhibit insignificant reductions in metabolism, so possible increases in oxygen needs in freshwater can not be compensated for in this manner. Furthermore, a number of studies have indicated that Hb-O_2 affinity is decreased in freshwater conditions (e.g., Benditt et al., 1941; Weber et al., 1976; Woo and Wu, 1982). Thus, oxygen carrying capacity may have to be increased in quantitative ways if oxygen affinity of the hemoglobin molecule is decreased in dilute media.

Another finding from the present study was that *C. variegatus* exhibits little change in quantitative measures of oxygen carrying capacity over the range of salinities between 2 ppt and 50 ppt. Previous studies have shown that salinities within this range have little effect on the metabolic rate, P_c, and osmoregulatory ability of *C. variegatus* (this study, Chapters 2, 3, and 4). However, as oxygen needs would be expected to rise as salinity was increased over this range, oxygen carrying capacity was expected to increase as well.

Several possibilities may explain why blood oxygen carrying capacity did not increase over this range of salinities. First, increases in salinity may require little compensation in oxygen carrying capacity until extreme hypersalinities are reached. However, it is more likely that the lack of a response may have been due to the fact that

large variations in salinity alone seldom occur under natural conditions. A larger response would be expected if salinity were varied together with oxygen and/or temperature. Another possibility is that *C. variegatus*, like many fish species, may utilize multiple hemoglobins. Weber (1990) noted that the use of multiple hemoglobins can extend the range of conditions under which oxygen is transported efficiently, thus enlarging the range of available habitats. If *C. variegatus* utilizes such a mechanism, large quantitative changes in oxygen carrying capacity might not be seen.

Finally, *C. variegatus* may exhibit mostly qualitative changes in response to salinity, i.e., the primary response to changing salinity may be better reflected in Hb-O_2 affinity. Changes in Hb-O_2 affinity as a result of environmental changes are extremely common in fishes (e.g., Johansen and Weber, 1976; Wells et al., 1980; Weber, 1981), although this has rarely been examined with respect to salinity. However, studies on *Anguilla anguilla* (Weber et al., 1976) and *Salmo salar* (Maxime et al., 1990) did find that increases in salinity between freshwater and seawater led to increases in Hb-O_2 affinity. A high affinity hemoglobin molecule might also be advantageous under hypersaline conditions, although it may be ineffective during activity (McMahon, 1988). As Hb-O_2 affinity was not measured in this study, direct correlation with *C. variegatus* is purely speculative at this time. Nevertheless, changes in Hb-O_2 affinity would correlate well with the large decreases in energetic expenditures exhibited by *C. variegatus* at high salinities. However, other factors could also be involved. First, *C. variegatus* may exhibit low salt sensitivity over the normal range of salinities encountered to reduce dependence of blood oxygen affinity on environmental salinity (Weber et al., 1976). Second, the primary mechanism used to alter Hb-O_2 affinity results from swelling of erythrocytes (Jensen, 1991; Wells and Weber, 1991; Jensen et al., 1993). Swelling of erythrocytes is normally accompanied by a rise in cell pH and decreased red cell hemoglobin, NTP and ATP concentrations, all of which serve to increase the oxygen affinity (Wells and Weber, 1991). However, as seen by the MCHC and MCV values determined in this study, erythrocytes did not swell in response to

increased salinity in *C. variegatus*. Thus if Hb-O_2 affinity is altered with changes in environmental salinity, it must be changed in some other manner.

This study clearly indicates that salinity does influence the oxygen carrying capacity of the blood of *C. variegatus*. Quantitative differences in hemoglobin concentration, hematocrit, and erythrocyte count were noted in response to changing salinity. As discussed above, it also seems likely that salinity may influence qualitative changes in blood oxygen transport in *C. variegatus*. Further research is needed to better understand the influence of salinity on blood oxygen levels in euryhaline teleosts.

CHAPTER 6
SUMMARY AND CONCLUSIONS

This study examined costs associated with life of a teleost in a variable salinity environment, represented here by a salt marsh. *Cyprinodon variegatus* was used to examine the influence of salinity on routine metabolic rate (RMR), critical oxygen tension (P_c), osmoregulation, and blood oxygen carrying capacity. Results are summarized below.

1) Field measurements in the Cedar Key salt marsh indicated that this habitat undergoes extensive variation in salinity, temperature, and oxygen.

2) RMR was relatively constant over a range of salinities from 0 ppt to 40 ppt. At higher salinities RMR began to decline, and was significantly depressed under hypersaline conditions.

3) Following sequential acclimation to experimental salinities, P_c was unaffected by changes in salinity between 0 ppt and 40 ppt, with P_c increasing at higher salinities.

4) Reduction in metabolism and rise in P_c corresponded well with a reduced ability of *C. variegatus* to regulate plasma osmolality efficiently. Osmotic permeability of the gills may be reduced at high salinities to offset osmotic losses or ionic gains to/from the environment, indirectly reducing the potential for oxygen uptake as well.

5) Variations in RMR and P_c as a function of environmental salinity observed in this study suggest that *C. variegatus* responds to high salinities by reducing energy expenditures. These responses effectively increases the time *C. variegatus* can tolerate such conditions, albeit at a cost of a reduction in energetic processes. This strategy fits the concept of scope for survival, as described by Hochachka (1990).

6) When *C. variegatus* was exposed to simulated tidal changes in salinity, RMR was unaffected in salinity trials where both acclimation and final salinities were in the range

typically encountered by this population in its native habitat. Where the acclimation or final salinities were extremely high (50 and 60 ppt) or extremely low (0 ppt), RMR was depressed.

7) Acclimation state was the most important factor determining the metabolic response to simulated tidal changes in salinity. However, direction of the salinity change also influenced metabolism in *C. variegatus*, with increasing salinity dealt with more efficiently than decreasing salinity.

8) Simulated tidal experiments corroborate the hypothesis that *C. variegatus* tolerates extremes in salinity by lowering metabolism, and hence decreasing energy expenditures. Following adverse conditions metabolism returns to normal levels.

9) *Cyprinodon variegatus* is an excellent regulator of plasma osmolality even when exposed to large fluctuations in salinity within the range of salinities typically encountered. Daily fluctuations in salinity of up to 30 ppt elicited no significant differences in osmoregulatory ability when compared to control fish.

10) Prior exposure to fluctuations in salinity does impart an osmoregulatory advantage. Fishes previously exposed to large fluctuations in salinity regulated plasma osmolality better than fishes that had previously experienced no or small changes in salinity. Increasing salinity had a greater impact on regulation of plasma osmolality than did decreases in salinity.

11) Salinity had a significant effect on blood oxygen carrying capacity in *C. variegatus*, although differences were only noted at the very highest (60 to 80 ppt) and lowest (0 ppt) salinities tested. Oxygen carrying capacity and all blood indices were highest in the group acclimated to 0 ppt.

12) *C. variegatus* exhibited little change in oxygen carrying capacity over the range of salinities between 2 ppt and 50 ppt. Possible reasons for this include: (a) increases in salinity may require little compensation in oxygen carrying capacity; (b) detectable changes may only occur when salinity is varied in conjunction with variations in oxygen and/or

temperature; (c) *C. variegatus* may utilize multiple hemoglobins, and/or; (d) the primary mechanism to increase oxygen carrying capacity may instead be through adjustment of Hb-O_2 affinity.

13) Erythrocyte count was the most consistent and hematocrit the least consistent measure of the influence of salinity on blood oxygen level.

Competition or predation pressure may be less intense in harsh, fluctuating environments, and that certain species may avoid these pressures by evolution of wide physicochemical tolerances and the use of such environments (Matthews and Styron, 1981). *Cyprinodon variegatus* seems to fit this mold well, as this species appears to be a generalist that very successfully inhabits harsh and variable habitats where it does not have to be very efficient to compete with other species of fishes (Martin, 1972; Berry, 1987). This argument may explain why *C. variegatus* does not invade freshwater in more locales, and why they are not very abundant in most freshwater systems; except in south Florida freshwaters where temperature and dissolved oxygen are variable and extreme.

Furthermore, it is believed that *C. variegatus* has conquered its wide geographic range by physiological flexibility rather than by local accommodation in physiology or life history (Berry, 1987). This hypothesis remain to be rigorously tested, although this study lends further evidence for the physiological flexibility of *C. variegatus*. Examination of the physiological ability of other populations of *C. variegatus* may help to resolve this issue.

The success of a fish species in a stressful environment may depend primarily upon the proportion of the population that survives the adverse conditions, with even short-term increases in tolerance significant (Matthews and Styron, 1981). This hypothesis appears to be relevant to the results of the present study. *Cyprinodon variegatus* is well adapted to a varying salinity environment. Its metabolic rate, P_C, and ability to efficiently osmoregulate is unaffected by changes in salinity over the typical range encountered, even when salinity is changed very rapidly. Furthermore, *C. variegatus* appears to tolerate extremes in salinity by decreasing energy expenditures, waiting for conditions to improve,

and then responding by increasing metabolism back to normal levels. This is a potentially adaptive response for life in a variable salinity environment.

While this study answers many questions relating to the physiology of salinity adaptation in *C. variegatus*, it also raises new ones. Chief among these is the question of how responses due to salinity would be influenced by interactions with changes in temperature and/or dissolved oxygen. For example, *C. variegatus* normally experiences high salinities together with high temperatures. The interaction of these two parameters on osmoregulation, metabolism, and oxygen carrying capacity would be a natural continuation of the current study. Another area of future research would include an examination into the possible ability of *C. variegatus* to utilize anaerobic metabolism under varying salinity regimes, as it was suggested by Subrahmanyam (1980) that salt marsh fishes may utilize anaerobic metabolism more than other groups of fishes. It has also been proposed that permeability changes of the gill may lead to reductions in metabolism at high salinities (Nordlie et al., 1991). A histological study of the gills of *C. variegatus* acclimated to a wide range of salinities to study chloride cell recruitment and hypertrophy and changes in functional gill surface area may be used to help resolve this question. Finally, understanding the influence of salinity on oxygen carrying capacity requires research on Hb-O_2 affinity as a function of salinity.

Salinity is a crucial physicochemical factor that exerts an important influence on aquatic life. *Cyprinodon variegatus* is an extremely competent euryhaline teleost that can thrive in a wide variety of coastal habitats. The assertion by Davenport and Sayer (1993) that "fish that are capable of withstanding sudden and frequent salinity changes are generally specialized morphologically and physiologically in ways that restrict their lifestyle" seems erroneous when fishes such as *C. variegatus* are considered. While few data exist on the responses of other fishes to wide ranges in salinity, the patterns seen in *C. variegatus* may represent a general pattern for fishes inhabiting variable salinity environments.

APPENDIX 1
CRITICAL OXYGEN TENSION FIGURES

Critical oxygen tension (P_c) figures were plotted for each fish used in the study described in chapter 2. These plots consisted of weight-adjusted oxygen consumption rates (mg O_2 h^{-1}) plotted against oxygen tension (mm Hg) during the interval over which oxygen consumption was calculated. Because 111 fish were used in this study, it is impractical to show each figure. Figures A1-1, A1-2, and A1-3 are representative samples of these figures. Generalized P_c figures were produced for each salinity group from the mean P_c, mean routine metabolic rate (RMR), and mean slope in the conformation region. These are shown in figure A1-4.

Figure A1-1. Plot indicating the calculation of the critical oxygen tension (P_c) for an individual *Cyprinodon variegatus* in water at 0 ppt.

Adjusted Routine Metabolic Rate
(mg Oxygen/hr)
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
160
Oxygen Tension (mm Hg)
Pc = 62.0 mm Hg
Salinity = 0 ppt
Fish Weight = 1.94g

Figure A1-2. Plot indicating the calculation of the critical oxygen tension (P_c) for an individual *Cyprinodon variegatus* in water at 50 ppt.

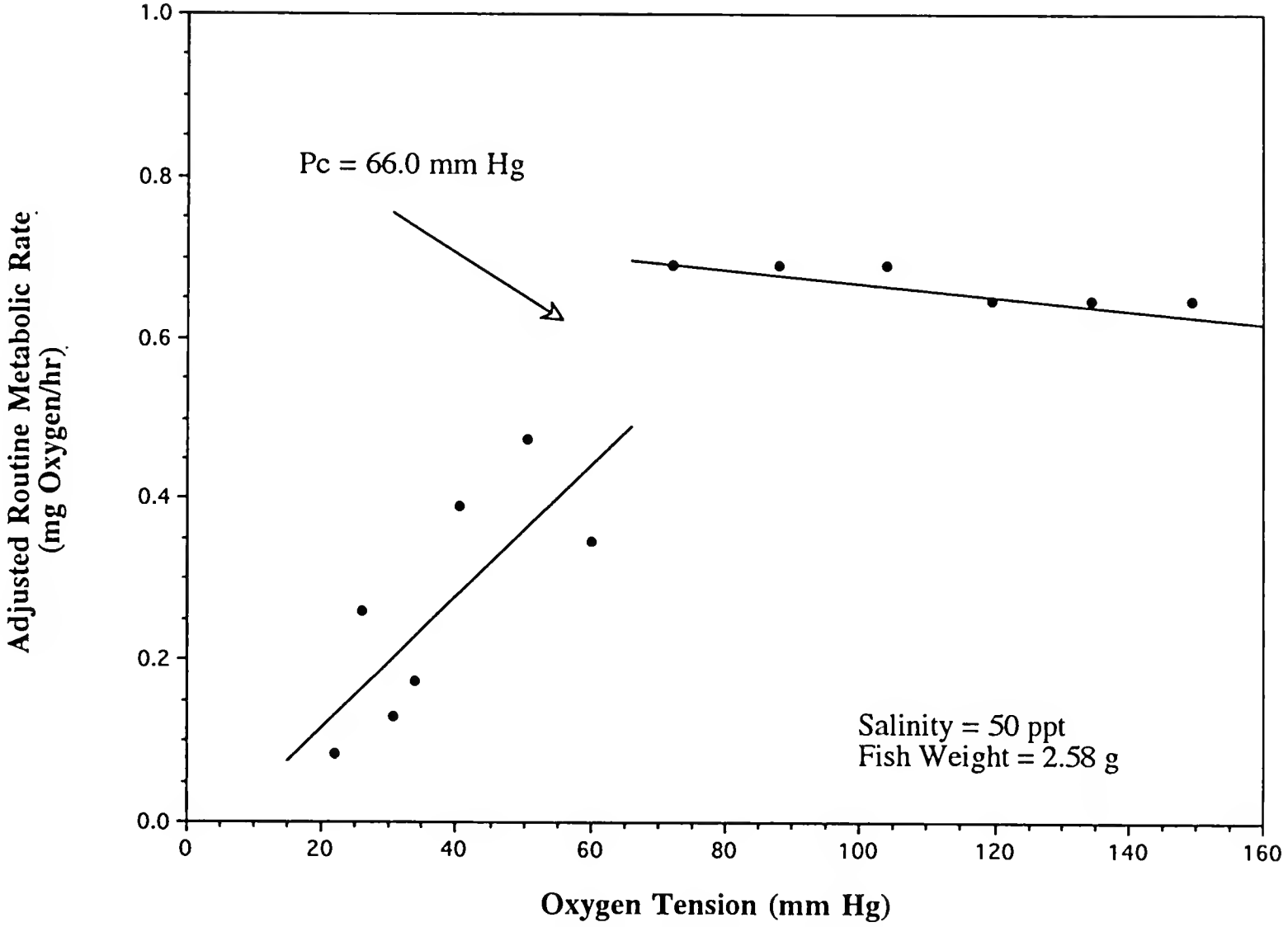

Adjusted Routine Metabolic Rate
(mg Oxygen/hr)
1.0
0.8
0.6
0.4
0.2
0.0
Pc = 66.0 mm Hg
Salinity = 50 ppt
Fish Weight = 2.58 g
0
20
40
60
80
100
120
140
160
Oxygen Tension (mm Hg)

Figure A1-3. Plot indicating the calculation of the critical oxygen tension (P_c) for an individual *Cyprinodon variegatus* in water at 100 ppt.

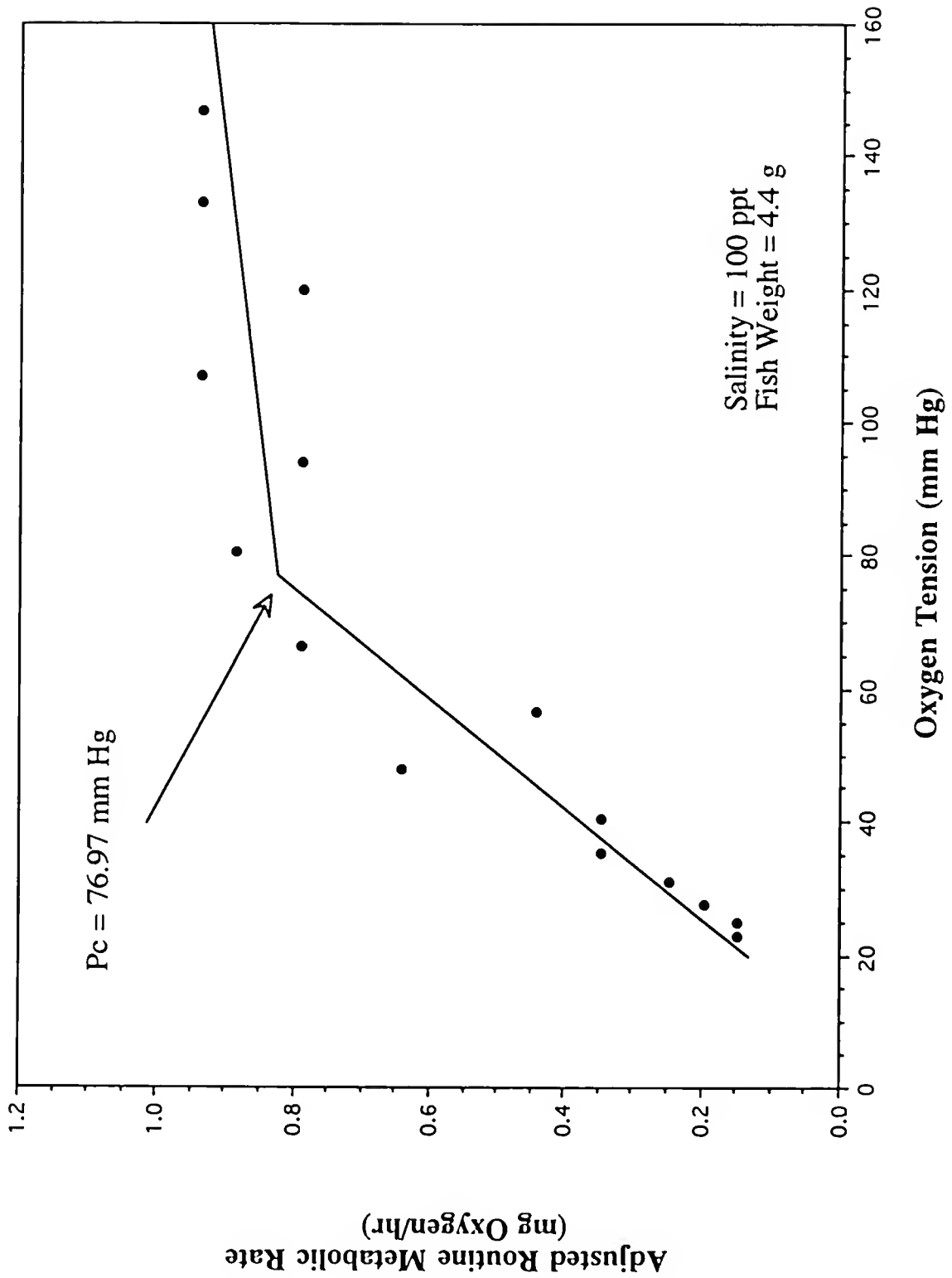

Adjusted Routine Metabolic Rate
(mg Oxygen/hr)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
160
Oxygen Tension (mm Hg)
Pc = 76.97 mm Hg
Salinity = 100 ppt
Fish Weight = 4.4 g

Figure A1-4. Generalized critical oxygen tension (P_C) plots at each salinity used in the P_C experiments. Plots were produced by using the mean P_C, mean routine metabolic rate (RMR), and mean slope in the conformation region for each salinity group.

0 ppt
Oxygen Consumption Rate
(mg Oxygen/hr)
Oxygen Tension (mm Hg)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
20
40
60
80
100
120
140
160

2 ppt
Oxygen Consumption Rate
(mg Oxygen/hr)
Oxygen Tension (mm Hg)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
20
40
60
80
100
120
140
160

15 ppt
Oxygen Consumption Rate
(mg Oxygen/hr)
Oxygen Tension (mm Hg)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
20
40
60
80
100
120
140
160

30 ppt
Oxygen Consumption Rate
(mg Oxygen/hr)
Oxygen Tension (mm Hg)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
20
40
60
80
100
120
140
160

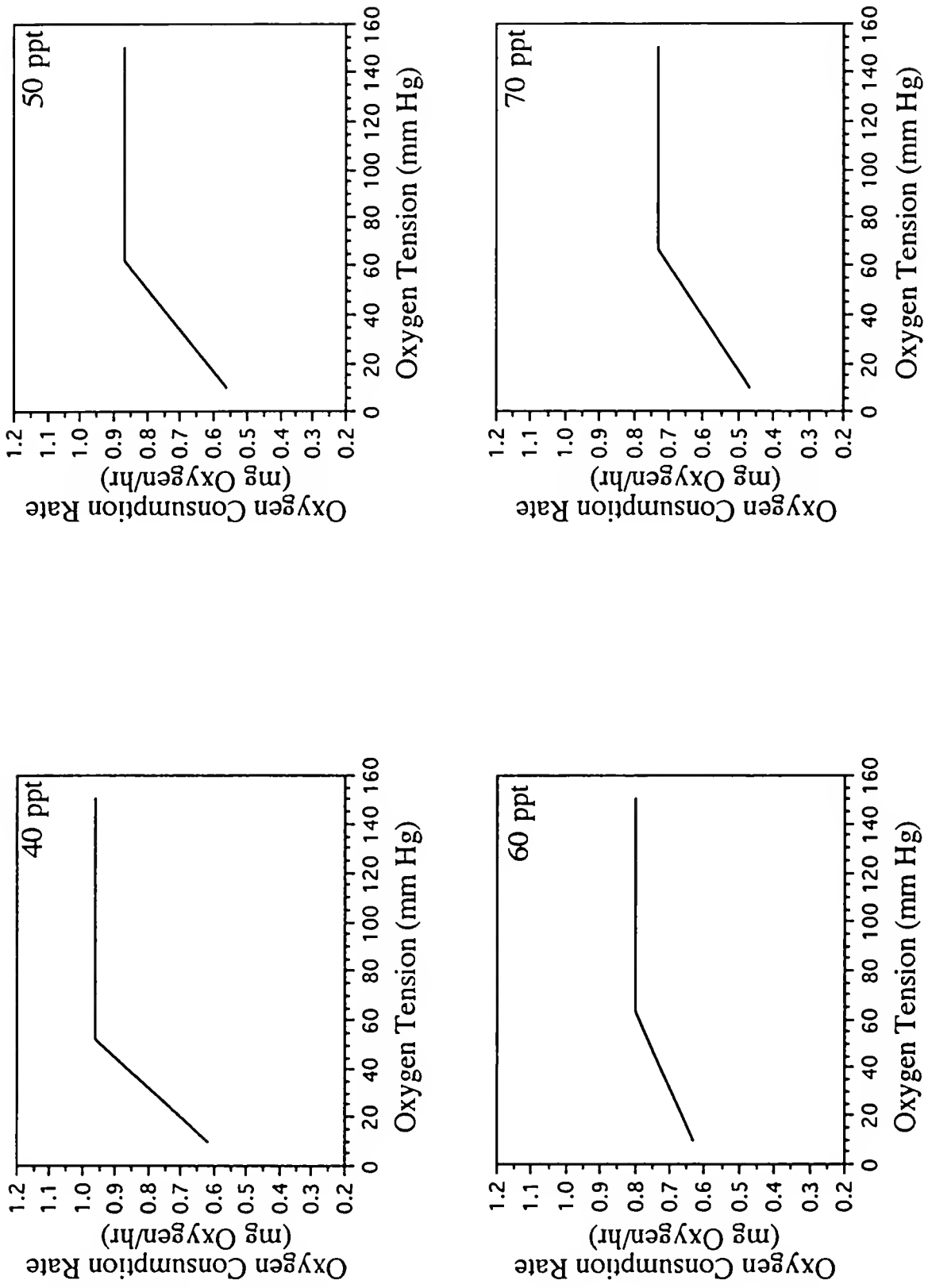
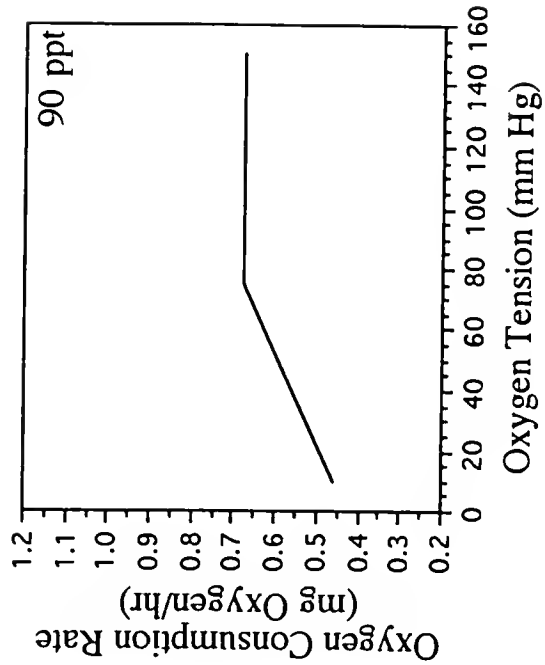
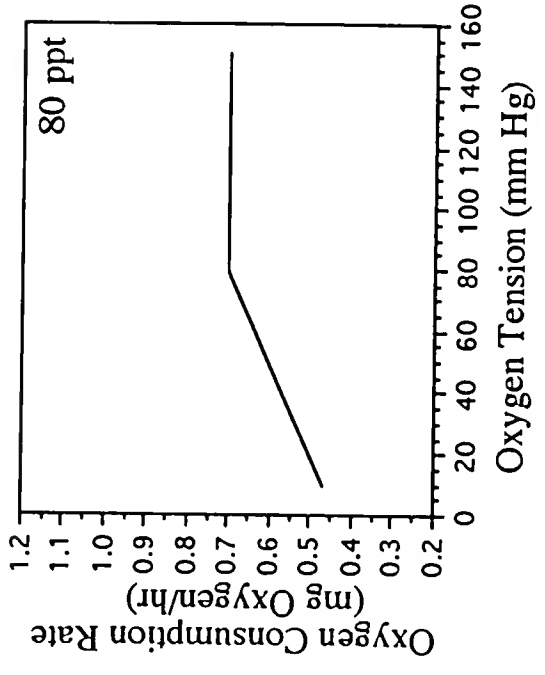


Figure A1-4 -- continued





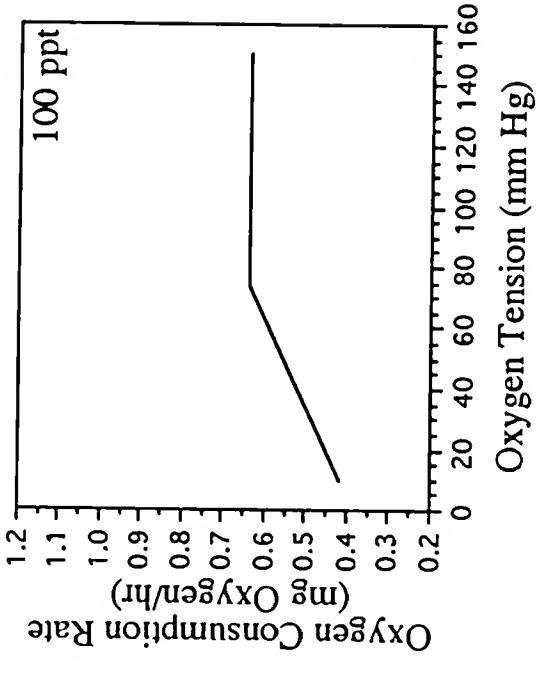


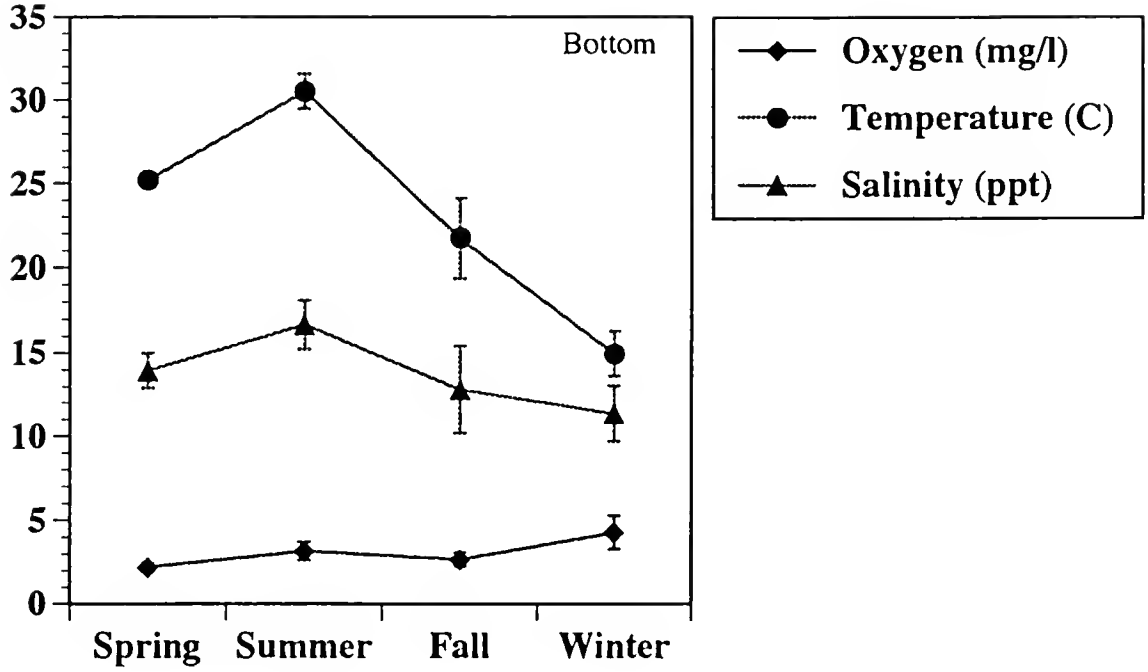
Figure A1-4 -- continued

APPENDIX 2
FIELD MEASUREMENTS

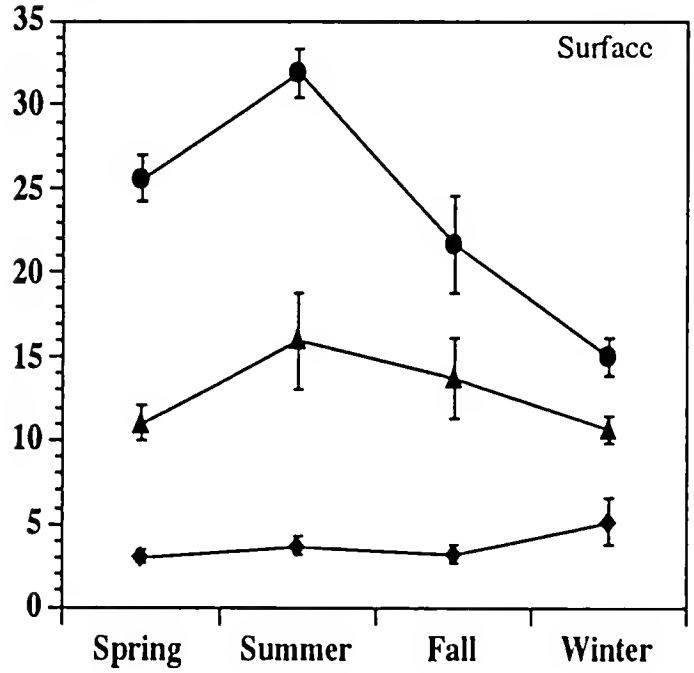
Field measurements were taken in the Cedar Key area from June 1990 through June 1991. These measurements were taken at two depths (bottom and surface) for each of four sites in the area, whenever possible. The variables measured were oxygen concentration (mg L^{-1}), salinity (ppt), and temperature (^{o}C). Figure A2-1 shows the relationships between these three variables at each site and depth over the course of the year. Oxygen concentration was highly inversely correlated with changes in both salinity and temperature. Salinity seems to be most highly correlated with changing oxygen levels, although salinity and temperature appear to be linked as well, albeit with more site to site variation.

Figure A2-1. Oxygen concentration (mg L^{-1}), salinity (ppt), and temperature (^{o}C) at four sites in the Cedar Key area taken between June 1990 and June 1991. Values given as means, bars indicate ± se.
a) Measurements taken on the bottom at site 1; b) measurements taken on the surface at site 1; c) Measurements taken on the bottom at site 2; d) measurements taken on the surface at site 2; e) Measurements taken on the bottom at site 3; f) measurements taken on the surface at site 3; g) Measurements taken on the surface at site 4.

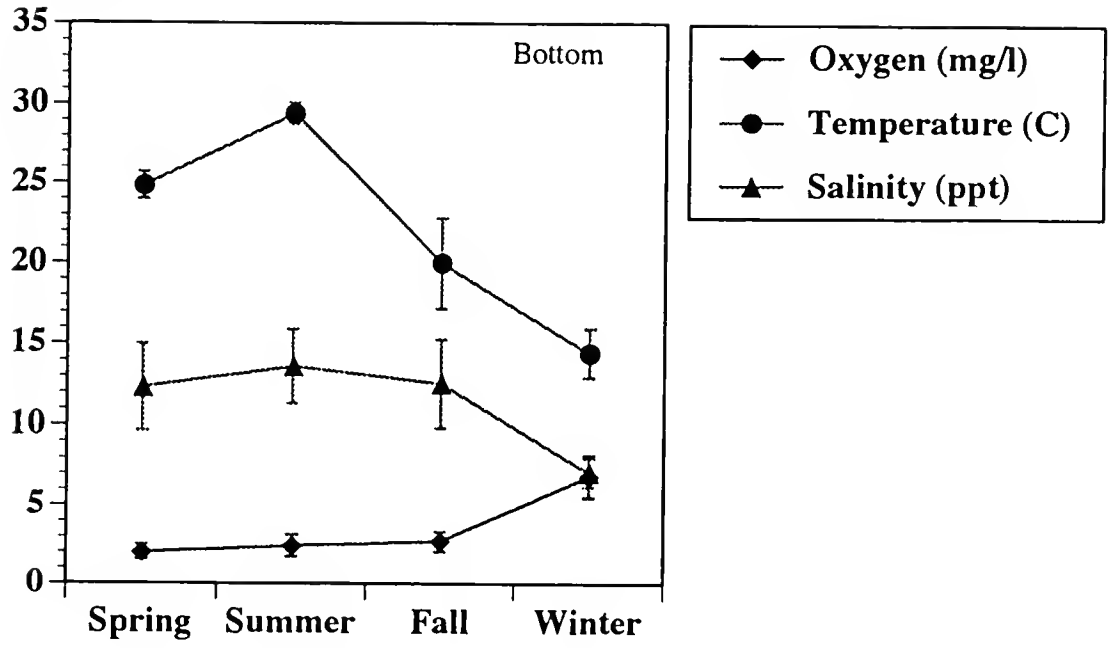
Part a


Bottom
35
30
25
20
15
10
5
0
Spring
Summer
Fall
Winter
Oxygen (mg/l)
Temperature (C)
Salinity (ppt)

Part b


Surface
35
30
25
20
15
10
5
0
Spring
Summer
Fall
Winter

Part c



Part d

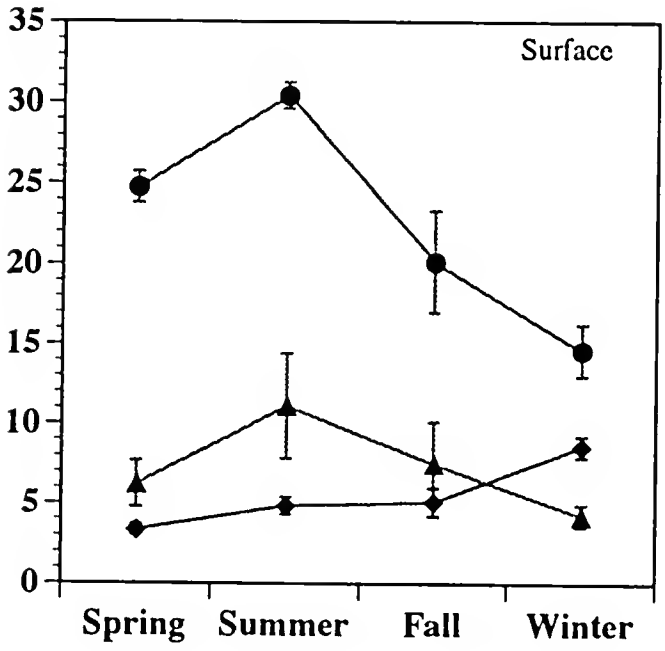
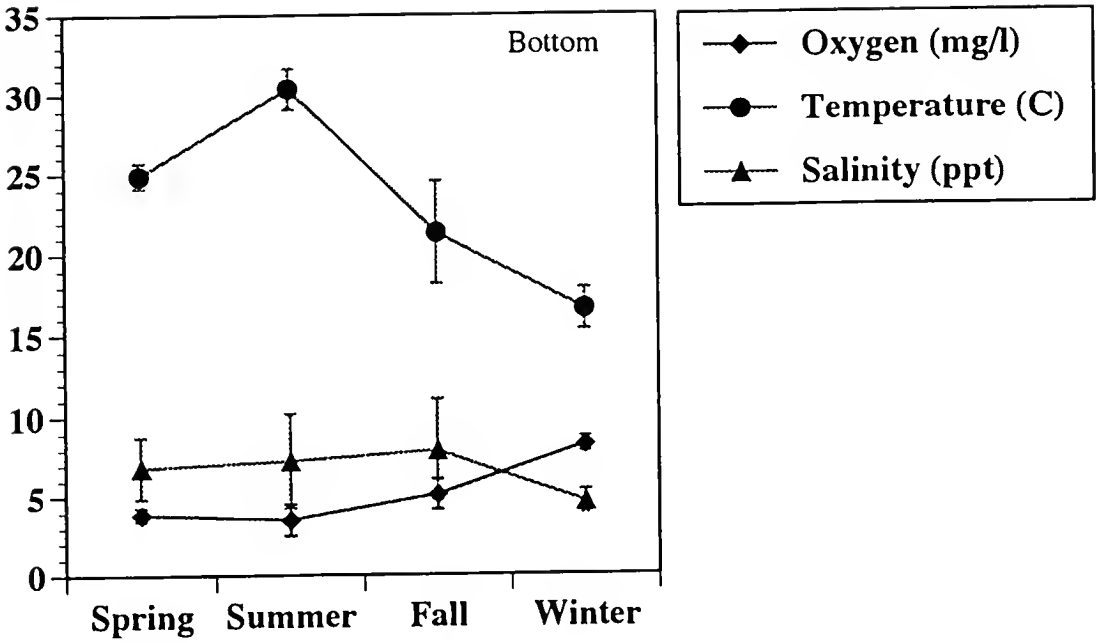


Figure A2-1 -- continued

Part e



Part f

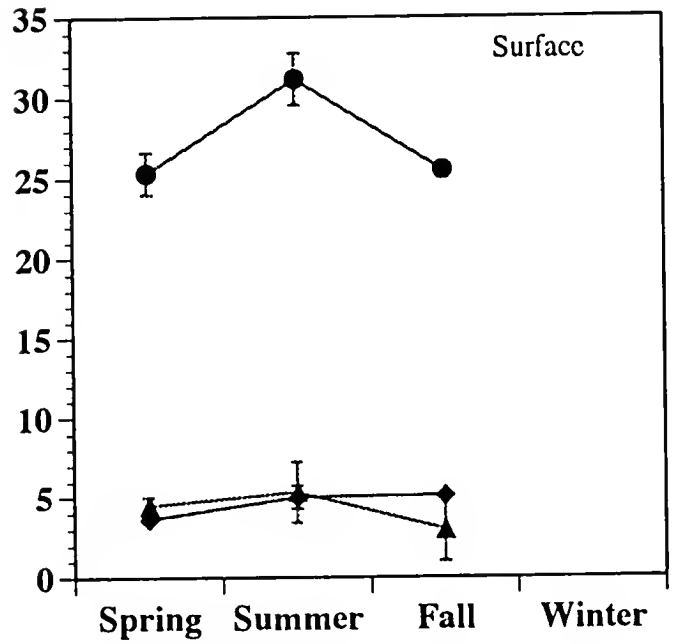


Figure A2-1 -- continued

Part g

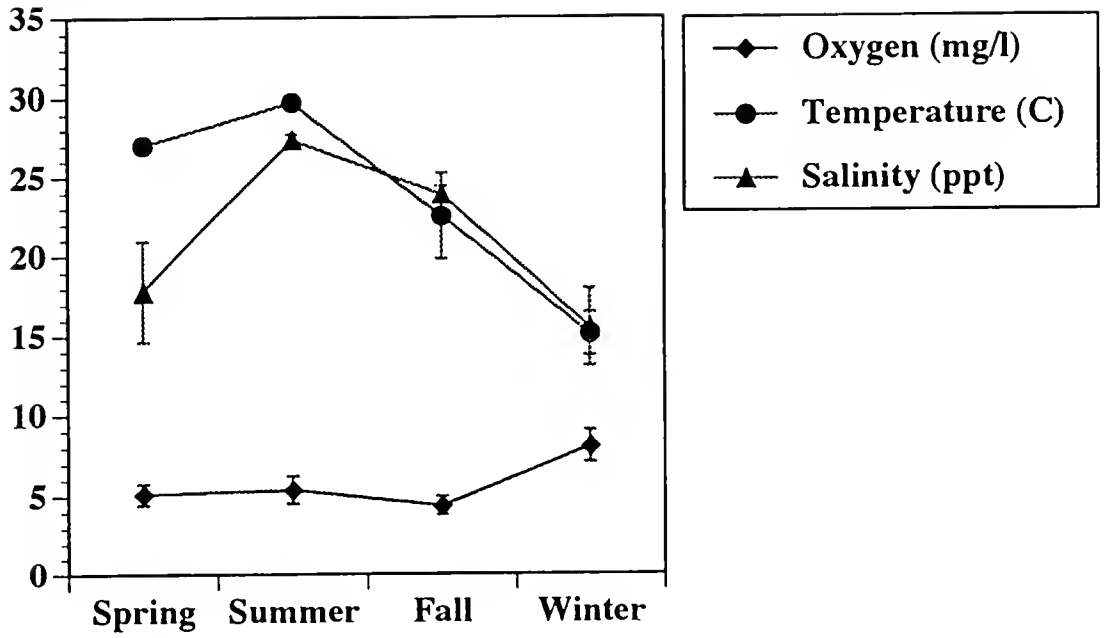


Figure A2-1 -- continued

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BIOGRAPHICAL SKETCH

Dennis Charles Haney was born March 18, 1962, in Inglewood, California to Charles and Jeanne Haney. He grew up in Southern California with his parents and older brother Scott, graduating from Chatsworth High School in 1979. Dennis began his career in the biological sciences as an undergraduate at the University of California, San Diego (UCSD). While at UCSD Dennis had his first true exposure to the joys (and pitfalls) of research and teaching. He graduated in 1983 with a B.A. in biology (specialization in animal physiology), and moved on to the graduate program at Oregon State University (OSU). Dennis spent two years at OSU, where he was introduced to the study of fish physiology. For his master's thesis he examined the physiological and hematological effects of erythrocytic necrosis virus on chum salmon (*Oncorhynchus keta*). Dennis completed his M.S. in biological science in 1985, following which he moved across the country to begin a doctoral program at the University of Florida (UF). Since 1990 Dennis has simultaneously worked on his dissertation and for the Department of the Interior as a Biological Technician. Dennis completed his dissertation and graduated from UF in 1995.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frank Nordlie, Chair
Professor of Zoology

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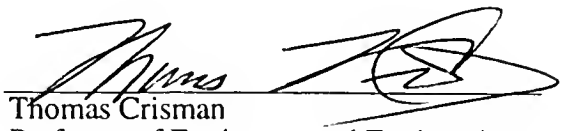

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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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